IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re patent application of Anderson et al

Confirmation No. 4972

Serial No. 10/788,413 Group Art Unit 1617

Filed March 1, 2004 Examiner Wang

For TREATMENT USING DANTROLENE

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF DAVID M. ANDERSON UNDER 37 C.F.R. 1.132

David M. Anderson declares as follows:

1. I am an inventor of the above-identified application. I hold a position in Lyotropic Therapeutics, Inc., the assignee of record of the above-identified application, as Vice President Scientific Affairs. I have read and understand the application, and I have read and understand the office action mailed June 12, 2008. I have also read and understand the references of record.

2. I am an expert in the fields of chemical and pharmaceutical formulations and drug delivery systems, including as to the preparation, stabilization and characterization of particulate suspensions, dispersions and lyophilized and reconstituted material, as well as structured fluids including emulsions, liposomes, lyotropic liquid crystals, including reversed cubic, reversed hexagonal phase materials, and the like, for all routes of administration, including but not limited to intravenous ("IV") and oral. As evidence of my expertise, I have attached hereto my curriculum vitae (CV) as **Attachment 1**. I hold the degree of Masters in Mathematics and Ph.D. in Chemical Engineering. I have

authored over twenty papers which appear in refereed journals, and I am a highly skilled investigator competent to conduct experiments on dispersions of particulate formulations, including microcrystals and structured fluids, and to utilize equipment for properly characterizing the nature of such materials. Based on my education, training and experience as set forth in the attached CV, I am qualified to provide opinion evidence on the level of skill of one of ordinary skill in the art, and as to what would be obvious or not obvious to one of ordinary skill in the art. In addition, I am qualified to conduct experiments and to provide test results relating to various chemical formulations for pharmaceutical purposes.

3. I have conducted experiments that, when interpreted in light of the established literature in the field, demonstrate that the formulations disclosed in Japanese Patent No. S53-20413 (Mitomi): (i) are not suitable for IV administration; and, (ii) the disclosure does not set forth any teaching for the preparation of formulations suitable for IV administration. These experiments flatly disprove the Examiner's conclusion that because Mitomi disclosed pharmaceutical formulations of some kind, such formulations were suitable for IV administration. In fact their administration by IV would probably be lethal.

4. At my direction and under my supervision and review, laboratory personnel at Lyotropic Therapeutics conducted the following scientific work and obtained the following data, all as set forth in **Attachment 2.**

A. We prepared Embodiments 1, 2, 4 and 5 of Japanese Patent No. S53 – 20413 exactly presented in that disclosure, namely, for:

> "Embodiment 1. 25 g sucrose, 850 mg sodium citrate and 200 mg methylparaben were dissolved in purified water, 500 mg of dantrolene sodium was suspended within the solution and purified water added to a total volume of 100ml."

The sodium dantrolene was obtained from a manufacturer with a DMF on file with the FDA. After the dantrolene add, the dantrolene did not fully go into suspension, and the

mixture was shaken by hand. Photos were taken at 15 seconds, 30 seconds and 5 minutes after shaking. Samples were taken for microscopy immediately after shaking.

(Note that Embodiment 3 was not prepared due to the lack of the excipients. However, given the similarity of excipients in Embodiment 3 with those in the other Embodiments, I know of no reason it should yield materially different results than Embodiments 1, 2, 4 or 5 over the observed parameters.)

B. Procedure. Each of the Embodiments, after preparation, was examined by me and lab assistants under a Polyvar brand light / DIC (Differential Interference Contrast) microscope, and photographed. Vials of each Embodiment were examined visually for the speed and extent of settling, and the results photographed. Settling is demonstrated by a change from homogenous color and density of material to two or more regions of differing color and density material. Dantrolene is bright orange. Thus settling is indicated both by the obvious enhancement of color in one region, and the diminishment of color in the other.

C. Findings. Each of Embodiments 1, 2, 4 and 5 resulted in a high density of particles greater than 5 microns. In addition, each showed large numbers of extremely large (over 10 microns) aggregates of particles. These were readily visualized in the microscope: the micrographs show multiple large particles and aggregates per field. This result was confirmed by "settling" analysis. In each case, within the timescale of a few minutes the material in vials separated into two distinct regions - an upper region relatively clear and transparent, and a lower region bright orange and opaque with visible solid material. The dantrolene particles and aggregates were so large they sunk quickly in the aqueous medium.

5. On a later date, we again prepared Embodiments 1, 2, 4 and 5, however, this time, adding additional procedures not disclosed in the disclosure, namely, homogenizing the mixture and, ultimately, subjecting the mixture to high speed microfluidization, in order to obtain full dilution and reduce particle size. We homogenized at 10,000 rpm for 15 minutes. We took photographs and viewed under the DIC microscope as before. Results were virtually unchanged. We then repeated for Embodiment 1 substituting a larger and

more forceful homogenizer (Silverson) for the additional step, and again viewed under the microscope and photographed.

6. Finally, for comparison with a formulation of the Instant Invention, we prepared material according to Example 1 of the Instant Invention, and then prepared micrographs of the formulation and photographs of vials, examining for settling and other evidence of stability. These are provided in **Attachment 3**. In the micrographs, no particles over 5 microns are visible, and no aggregates whatsoever are visible. The photographs of vials show, after comparable time periods, no observable differentiation of regions, and thus no indication of any settling whatsoever. As indicated in the Instant Application at Example 1, the conclusion that there are virtually no large particles in the invention is confirmed by data obtained from analysis of the sample by the Beckman-Coulter N4 Plus PCS particle size analyzer instrument, due to the high sensitivity of PCS to larger particles. By contrast, Embodiments 1, 2, 4 and 5 prepared according to Japanese Patent No. S53 – 20413 could not even be analyzed by the particle sizer instrument because the particles and aggregates were so large they are outside the operative range of the instrument.

7. Conclusions: Every one of the Embodiments of Japanese Patent No. S53 – 20413 which was prepared by common methods is unsafe for IV injection. This was true even after we took the undisclosed additional step of homogenization and microfluidization—neither of which were, in any case, mentioned or alluded to in S53 - 20413. As indicated in the Burgess reference supplied with a previous filing in this case, and as is well known to those skilled in the art, the particle size of injectable formulations above 3 -5 microns are to be avoided because they cause pulmonary emboli. Even smaller particles are required when the particulate matter is rigid (as are particles of the solid crystalline material dantrolene) as compared to flexible (as an oil-in-water emulsion droplet, for example). The micrographs, the known physical properties of dantrolene, and the very high shear required to break down particle size all underscore the high rigidity of dantrolene crystals. The Embodiments contain a high concentration of particles far

greater in size than is accepted as safe for injection, and massively large aggregates of such particles, and are patently unsafe for injection. On this evidence, these particles would be perfectly suitable for an oral formulation, as the Japanese disclosure itself states they are "as the present invention is for ordinary oral administration". The absence of the disclosure of stabilizers means that the particulate matter will continue to aggregate over time, increasing the number of very large particles in the formulation, well into the range known to be deadly in IV formulations, but not inconsistent with oral formulations.

8. Therefore, it is my opinion, based on the experimental results noted above (which one of ordinary skill in the art would be able to duplicate), based on the level of skill of those of ordinary skill in the art, and based on the divergent subject matter described in the associated documents, one with ordinary skill in the art would be aware that naively injecting a drug formulation designed for oral administration is fraught with dangers, and is in fact life-threatening. This is absolutely true in the case of the Embodiments of S53 – 20413.

9. In my opinion, as one of ordinary skill in the art of preparing and characterizing pharmaceutical formulations, the Examiner's statement that any pharmaceutical composition disclosed should *ipso facto* reasonably be expected to be safe for IV injection is erroneous. It is well known in pharmaceutical development that standards for intravenous products are quite different than standards for pharmaceutical products administered by other routes of administration (See, for example, Injectable Drug Development, Pramod K. Gupta and Gayle A. Brazeau, Ed., Chapter 1, Challenges in the Development of Injectable Products, by Michael J. Akers), and "make injectable drug formulation, processing and delivery so complicated compared to other pharmaceutical dosage forms." These include the relatively fewer safe and acceptable formulation additives that can be used to solve the various formulation problems which may arise, such as solubility, stability, isotonicity, … "Because of the irreversibility of the injectable route of administration and the immediate effect and the contact of the drug product with the bloodstream and systemic circulation,…" These include hypersensitivity reactions,

particulate matter, phlebitis, infiltration and extravasation and thrombosis. Particle size in excess of a certain size can be deadly. This is plainly obvious to one of ordinary skill in the art, particularly with respect to oral formulations and injectable formulations. I am aware of countless examples of oral formulations which would be unsuitable, even lethal, upon IV administration. The Japanese Patent No. No S53 – 20413 does not speak to these distinctions, such as particle size, osmolality, etc., except to specify that certain excipients may be incorporated into the disclosed formulation. However, the list of such excipients identifies some which are clearly unacceptable for IV administration.

10. In my opinion, the MH pig model is definitive model not only for efficacy of MH prophylaxis or treatment, but also for IV safety, as pigs are well known in the art to be quite sensitive to pulmonary emboli, the leading indicator of particle size problems with IV pharmaceutical administration .

11. In my opinion, the virtually immediate availability (within one minute by one person, as compared to 20 to 75 minutes by multiple persons) of an initial full therapeutic dose this emergency life-saving drug which results from the nearly instant and vastly simplified completion of reconstitution of stable lyophilized material and administration of small volume, due to its highly concentrated and stabilized nature, well within acceptable particle size limits, of the formulations disclosed in this invention is completely unobvious over the prior art, and of extraordinary utility.

12. I have very carefully reviewed the article, Intravenous Lecithin-Coated Microcrystals of Dantrolene are effective in the Treatment of Malignant Hyperthermia: An investigation in rats, dogs, and swine, Karan, Lojeski, Haynes et al, Anesth Analg 1996; 82:796-802 ("Article"). The Article does not put forth a safe for injection high concentration low volume formulation of dantrolene, and does not set forth a path along which the preparation of such a formulation could be achieved or would be obvious.

13. The Article posits and the Examiner apparently accepts that these are two different formulations, "MC-NaD" (in which the dantrolene is in the sodium form) and "MC-D" (in which the dantrolene is in the "neutral", i.e., "free acid", form), and that the difference is significant, and therefore the data which shows inescapably that MC-NaD is unsafe should not be seen as reflecting on the MC-D formulation. I disagree with this conclusion and believe it is unwarranted based on the science reported in the Article.

14. The Article clearly is intended as the debut of a new lecithin-based microparticle coating system, which is the central star of the story. [Page 796, Column 1, and Footnote 1 to US Patent 5,091,188 to Haynes]. The coating system is identical in composition and in the manner or preparation in the two "formulations". [Page 797, Column 1]. The difference in the two formulations is that MC-NaD is comprised of dantrolene in the sodium salt form, and MC-D is comprised of dantrolene in the neutral, or free acid, form. (Note: When the term "neutral" is applied to dantrolene in the Karan et al. paper, it refers to what is more commonly [and more accurately] called the "free acid" form, which is the term used in the Instant Invention. This term "free acid" distinguishes it from all salt forms, such as dantrolene sodium, dantrolene potassium, dantrolene ethanolamine, etc. In these salt forms, the dantrolene molecule is in its charged, anionic form, and thus it contrasts with free acid case, where the dantrolene is [or can be, depending on pH] substantially uncharged [hence the adjective "neutral"]. Karan's use of "neutral" does not mean that the compound is neutral in the sense of pH; indeed, the free acid form is, of course, acidic, not neutral in the pH sense.)

15. The source of the difference, offered by the Article, despite the identical coating, is that "...MC-NaD's tendency to aggregate was the result of a large dipole moment of the anionic form of dantrolene..." [Page 801, Column 2]. However, this makes little sense upon examination. The particle surface properties such as zeta potential are determined, quite naturally, by the outer surface component, which is lecithin, not dantrolene. Furthermore, as it is well known in the art that lecithin coatings are anionic (due to free fatty acids and acidic lipids). The additional anionic charge due to the anionic form of

dantrolene therefore would increase electrostatic repulsions between particles, which would cause a decrease in aggregation in the MC-NaD case.

16. Taken as interchangeable formulations, MC-NaD and MC-D yield data revealing that lecithin coated microcrystals of dantrolene have not been shown to be safe; just the contrary, as I will discuss below. Thus, for example, the statement on Page 801 that "From the vantage of safety, MC-D is superior to MC-NaD" does not by any reasonable criterion mean that MC-D is safe for injection on an absolute basis (particularly when applying the definition of "safe for injection" given in our application). Applying scientific rigor to the publication, and untangling the collection of incomplete and poorly described data, reveals that the data provided on the MC-D formulation are insufficient to prove, even cursorily, safety and efficacy of MC-D as per the stringent and safety-demanding requirements for intravenous pharmaceutical products.

17. Even assuming the distinctions between the two formulations are material and substantial, nevertheless neither is shown in the Article to pass for a safe-for-injection formulation.

18. MC-NaD. The Article itself admits that the MC-NaD formulation is unsafe due to pulmonary response that includes markedly elevated PAP and the fatal "cardiovascular collapse that was refractory of all resuscitation efforts" of a healthy swine. The fatal dose was a 0.15 mg/kg IV bolus, which is an order of magnitude less than the recommended dose of 2.5 mg/kg. [Page 799, Columns 1,2]. The Article states that mechanical filtration of the formulation should have rendered MC-NaD formulation safe, however, in fact it did not. "Larger diameter particles appear to be responsible for the pulmonary response, but the exact mechanism is unknown. In theory, filtration should remove the larger particles that can be present as a byproduct of the manufacturing or the reconstitution process. However, filtration was not successful in eliminating the pulmonary response of MC-NaD in swine." [Page 801, Columns 1-2.] In fact, MC-NaD was unsafe for injection under every circumstance. "Once it became evident that the

pulmonary response to MC-NaD could not be overcome by filtration or reformulation, experiments designed to determine its efficacy in the prevention or treatment of MH were stopped." (emphasis supplied) [Page 802, Column 1].

19. MC-D. The Article admits that MC-D is unsafe in the absence of filtering after reconstitution. "... when it became evident that filtration would be necessary for the safe administration of MC-D, further experimentation was halted until a custom filter design was finalized." (emphasis supplied) [Page 801, Column 1.]

20. The Article asserts that MC-D is safe after mechanical filtering after reconstitution. However, there is insufficient data to support this assertion in any meaningful sense. The swine model is understood among those skilled in the art to be the preferable animal model for pulmonary emboli, and there are specific and well known disadvantages to the dog model. The total number of swine administered unfiltered MC-D was 3. The Article itself, in another context, admits that given the small number of animals examined, no firm conclusions can be made. [Page 801, Column 2] A sample size of N = 3 is simply insufficient upon which to make any meaningful conclusions. Measurements are such, given this tiny N and wide variability, that with significant N, there would arise unsafe results even after filtering.

21. (In terms of demonstrating that the MC-D formulation does not satisfy the "safe for injection" definition given in the instant application, one could apply standard statistical methods to the data given in the final row of Table 2 [Page 801], to show that with a larger sample size, there is a high likelihood that more than 10% of treated pigs would experience life-threatening complications, as quantified by PAP value increases that are on a par with those in the first and third rows of Table 2—which by the authors' own admission are associated with life-threatening pulmonary emboli). Furthermore, one critical row of Table 2 shows administration in 20 smaller injections spaced at two minutes, for a total administration time of 40 minutes. It is obvious that spreading out the administration with such a protracted delivery would tend to mask the detection of

pressure increases caused by emboli that otherwise would be manifest in a more rapid administration consistent with the aims of the work.

22. The Article states the reason that MC-NaD is unsafe is because of undissolved crystals of dantrolene being released from the lecithin shell. "Our data suggest that the pulmonary response to MC-NaD was the result of undissolved dantrolene being release[d] [sic] from MC-NaD." [Page 801, Column 1.] Free Acid, or neutral, dantrolene, is significantly less soluble than sodium dantrolene (conservatively speaking, by one order of magnitude). If undissolved sodium dantrolene is, as the authors state, the cause of the unsafety, then released, undissolved free acid dantrolene will be more unsafe in this regard, as it will dissolve less quickly and less thoroughly, making dangerous emboli more likely.

23. There was no data that the filter necessary to make a safe product was ever created, nor on the results of its use. "... when it became evident that filtration would be necessary for the safe administration of MC-D, further experimentation was halted until a custom filter design was finalized." [Page 801, Column 1.]. The requirement of "safe for injection", which is necessary though not sufficient for a viable human-use formulation, has demands for consistency in production and properties, most fundamentally particle size.

24. MC-D was never administered to an MH animal, either in prophylaxis model or treatment model. Only MC-NaD was administered, and was shown to be somewhat effective but completely unsafe for injection.

25. Because of the filtration, the experimenters state, they were unable to determine the post-reconstitution, post-filtration drug concentration. "...Lack of a standard commercial 2 micron filter limited our ability to determine a precise post-filtration drug concentration." [Page 802, Column 1.] This inability to determine post-filtration drug concentration altogether halted the investigators' efforts to conduct pharmacokinetic

studies in swine. Even more, it strongly suggests that the authors experienced a significant removal of crystals in the 2 micron filtration—which would mean that large particles were ubiquitous.

26. There is no data establishing that filtered MC-D is efficacious. The Article presents data showing that filtration caused loss of lecithin-coated particles of dantrolene, but there is no evidence of how much was lost, or the potency of the filtered material which was subsequently administered to animals, either in absolute terms or in relation to unfiltered MC-D. Note that filtered MC-D was never administered to MH susceptible swine, either prophylactically or as treatment for an induced MH crisis. Indeed, filtered MC-D was never administered to any species in any study of efficacy, including the twitch tension study used for other forms of the formulation examined in the Article. Thus, the Article presents no evidence that filtered MC-D was potent or efficacious. The only data shown with respect to filtered MC-D is on Table II, showing PAP results on 3 healthy swine.

27. In view of the above, it is entirely plausible that filtration of MC-D post-reconstitution resulted in the retention on the filter of a significant amount of dantrolene, thus removing it from the administered material, yielding a subpotent formulation that, solely because of its subpotency, demonstrated less negative PAP results in comparison to the unfiltered MC-D, but that if this filtered MC-D material indeed had been tested in an efficacy model it would have been shown to be ineffective.

28. For highly anisotropic particles, such as the typically needle-like dantrolene crystal (and consequently coated dantrolene crystals), particle size measurement is inherently imprecise, and filtration is inherently problematic. The bottom line, however, is that a particle which measures 700 nm in a particle sizer may very well be retained by a filter rated at 2 microns, and that there is good reason to believe that a significant fraction of dantrolene was retained by filtration.

29. I have reviewed the literature, and to my knowledge there has been a complete absence of any further publication for the past 12 years since the Article, thus the prior art contains to information that the filter problem was ever overcome and a safe for injection formulation achieved.

30. While the Article states stable suspensions of MC-NaD and MC-D were obtained [Page 797 Column 1], later the articles reports data fundamentally contradicting the conclusion. "Further observation at 200x dilution showed a tendency for MC-NaD to aggregate even after filtration." [Page 801, Column 2]. (emphasis supplied)

31. We prepared MC-NaD in accordance with the procedures set forth in the Article and monitored particle size and shape by a Beckman-Coulter N4 Plus particle-sizer and DIC microscopy (with photographic attachment), both methods we use in the regular course of our work with our dantrolene formulation, Ryanodex®. The results showed MC-NaD demonstrated rapid particle size growth and far larger particle size than our formulation. **See Attachment 4.**

32. We attempted in our laboratories to prepare MC-D in accordance with the procedures set forth in the Article, but could not do so. Our attempts to prepare MC-D material in this manner used with the same type of microfluidizer as reported in the Article, and a model in the same series (M110L, similar to the M110F in the Article, these being nearly identical in operation), operating at the same pressure, 14,000 psi, as reported in the Article. Despite multiple attempts, and despite subsequently adding pre-homogenization steps, dilution steps and sonication steps, our efforts each time resulted in the equipment clogging with thick viscous material, indicating gross aggregating, flocculation and very large particle size.

33. The Instant Invention is a complete and safe for injection formulation. No filtration is required after reconstitution. No post-reconstitution filtration was actually done in connection with the animal tests of record, and this has included several dozen swine and

many dozens of rats. It is not anticipated that post-reconstitution filtration will be a recommendation let alone a requirement of the marketed product. The batch sheets currently call for filtration of liquid inputs prior to combination to reduce bioburden, and of the complete formulation before lyophilization. The critical concern with microcrystals and nanocrystals is to ensure and demonstrate with complete confidence that they retain their size and resist aggregation post-reconstitution. The Instant Invention does so, and thus there is no need for post reconstitution filtering; whereas according to the Article, the formulations of the Article clearly do not, and this is a cause of their safety problems. Conclusive efficacy data is on the same formulation as to which there is conclusive safety data. There have been no instances in any study conducted of the LT formulations of pulmonary hypertension or pulmonary emboli.

34. The current, and we believe final, formulation of the Instant Invention is sodium dantrolene nanocrystal dispersion, stabilized by surfactants which: are non-ionic, do not form lamellar structures, and because they are water-soluble, do form micelles. All of these are features taught away from in Haynes, as set forth below.

35. The Instant Invention solves the problem unsolved by Karan (for a complete, safe for injection, high concentration low volume formulation) by a completely different an unobvious approach - the use of a soluble surfactant, not an insoluble coating. This is explicitly taught against by US Patent 5,091,188, describing the technology used to create the Karan formulation. US Patent 5,091,188 [Column 13, Line 55 to Column 14, Line 2]: "Selection of the Membrane forming lipids for coating.... The primary requirement is that the coating lipid be membrane-forming. This is satisfied by all lipids which, in the presence of excess water, make bilayer structures of the type which is well-documented for phospholipid vesicles or liposomes. This requirement is not satisfied by .... Non-ionic surfactants... A second requirement is that the lipid not have a proclivity for converting into micellar structures.: ....High stability of the coating material in membrane form is necessary to keep the drug material from rearranging into macroscopic crystals. This is one reason why non-ionic surfactants do not work well for my intended

purpose."

36. The water-soluble surfactants preferred in the Instant Invention do not meet these criteria, and actually contradict all the criteria given. Nor does the lecithin used by Karan satisfy the criterion for a preferred stabilized in the Instant Invention, since lecithin's solubility in water is far less than 5 mg/mL.

37. The sodium salt of dantrolene is far preferable for pharmaceutical use than the neutral (i.e., free acid) form. It is an order of magnitude more soluble. Thus, it will have greater speed and completeness of dissolution so as to: (i) reduce the risk that undissolved crystals will cause emboli; (ii) reduce size, so that large particles are not removed from circulation before dissolution, thus decreasing total available dose from an injection; and, (iii) increase dissolved concentration of dantrolene in blood, so administered dose will have more therapeutic effect. As for its longstanding use in Dantrium IV®: the sodium form is the form that is in all approved dantrolene formulations, and is thus the form that has a history of efficacy and safe use, pharmaceutical-quality production, and comprehensive documentation, all of which are totally lacking in the case of dantrolene free acid.

38. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Dec. 11, 2008
Date

David M. Anderson, Ph.D.

David M. Anderson

PROFESSIONAL HISTORY

1999 – present: **V.P. Scientific Affairs** at Lyotropic Therapeutics, Inc. Principal scientist and director of research and development staff efforts developing novel injectable (including in particular IV) and oral drug-delivery systems and associated pharmaceutical formulations and products. Responsibilities include conceptualization, design, developmental production, and chemical and physical characterization of new microparticle, including nanocrystals and lyotropic liquid crystalline, formulations, support and design of animal safety and efficacy testing of developmental formulations in collaboration with CRO's and industrial partners, and interactions between lipid systems and microparticles with biological systems. 10487 Lakeridge Parkway, Ashland, VA (W) 804-550-1280 (H) 804-798-9597.

1995 - 1999: **Principal Scientist** at SelectRelease, L.C. Experimental work and oversight of research and development activities, analysis and planning. Developed oral controlled-release pharmaceutical formulations based on synergistic combinations of lipids/surfactants and novel crystalline and polymeric coating materials, with particular focus on formulations for intestinal release. Pharmaceutical formulation work with photodynamic therapy agents led to successful animal tests demonstrating sustained release leading to tumor necrosis.

1991 - 1995: **Assistant Professor, SUNY Buffalo Biomaterials Dept. and Department of Oral Surgery,**; also adjunct faculty member (Research Assistant Professor) in Biophysics and Chemistry Depts. Research centered around controlled-release materials, and nanoporous polymers and hydrogels incorporating a wide range of polymers including novel polymerizable lipids and surfactants. Taught polymer, biophysics, and biomaterials courses. On the faculty of the NSF Industry/University Center for Biosurfaces (IUCB) which focuses on issues of biocompatibility, bioadhesion, tissue compliance, and biofilm characterization.

1987 - 1991: **Guest Researcher, Univ. of Lund, Sweden**, with Hakan Wennerström and Björn Lindman in Physical Chemistry 1 Dept., a world-renowned department in colloid and interface science. Research focused on lyotropic liquid crystals, and the polymerization thereof. Funded by NFR (Swedish NSF) and by STU (Swedish Board of Technical Development) and various industrial partners.

1986 - 1987: **Post-doctoral fellow with the Polymer Science and Engineering and the Mathematics Departments of the Univ. of Massachusetts at Amherst**, with E. L. Thomas and David Hoffman. Research focused on block copolymers, primarily the modeling of thermodynamics and morphology in complex 3-dimensional microstructures.

1982 - 1986: **Director of X-ray Scattering Facility at the Univ. of Minnesota.** While a graduate student, responsible for all matters concerning the operation of the lab, which housed a Siemens D-500 Diffractometer with computer interfacing, and a modified Kratky small-angle camera with a position-sensitive detector. Instrument maintenance, training of users, billing and records, data analysis.

GRADUATE EDUCATION

Ph. D. Chemical Engineering, University of Minnesota, June 1986.
Advisors: H. Ted Davis and L. E. Scriven; Enhanced Petroleum Recovery/Surfactant Microstructures Group.
Thesis: "Studies in the Microstructure of Microemulsions".
M. S. Mathematics, University of Minnesota, May 1982.

AWARDS, AFFILIATIONS AND TECHNICAL SKILLS

Awards and societies: Graduation with high distinction; Tau Beta Pi, Phi Kappa Phi; Runner-up in 1993 Niagara Frontier Inventor of the Year Award; Finalist in 1997 Richmond's New Technology of the Year; Runner-up in 1988 "Innovation Cup" invention contest sponsored by a Swedish technical newspaper (Dagens Industri); member American Association of Pharmaceutical Scientists (AAPS), and American Chemical Society (ACS); Strathmore's Who's Who;

Virginia Science Resource Network, Virginia Academy of Science, National Directory of Scientific Experts.

Instrumentation skills. TEM/SEM, ultrafiltration (including hardware/system design), small- and wide-angle X-ray diffraction, pulsed-gradient NMR, aerosol monitoring, particle characterization with light scattering as well as aerosol techniques, polarizing, fluorescence and DIC optical microscopy, IR, NMR (chemical shifts), UV, electrophoresis and liquid chromatography. Aerosol generation and characterization expertise includes condensation particle counters, differential mobility analyzers, electrospray nebulizers. Strong background in the characterization of submicron/nanoscale particle characterization via a range of techniques, including particle sizers, zeta potential, microscopy, XRD, etc. Short course certifications in rheology, DSC, XRD, and laser particle sizers. Excellent skills in optical instrumentation such as microscopes and telescopes including design of new optical measurement techniques.

Mathematics/modeling/computer skills. Strong computing skills ranging from PC's to mainframe supercomputing, including innovative state-of-art graphics dating back to the earliest days of computer graphics. Sophisticated finite element analyses, including 3D graphics representations of solutions, supported by direct analytical calculations including published methods for a wide range of applied mathematics problems relating to nanostructured materials. Analytical and FE solutions of flow patterns, diffusion profiles, scattering/diffraction phenomena, etc. Modeled structure-property relations in polymers. Modeling of thermodynamics, microstructures, spectroscopic and other measurements of complex structures.

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D. M. Anderson, D. C. Martin, and E. L. Thomas, "Maximum entropy data restoration using both real and Fourier space analysis", **Acta Cryst.**, 1989 A45, 686-698.

D. M. Anderson, H. T. Davis, L. E. Scriven, "Mean and Gaussian curvatures of the randomly-decorated Voronoi and cubes tessellations", **J. Chem. Phys.**, 1989 91 (5), 3246-3251.

B. Lindman, Kozo Shinoda, U. Olsson, D. M. Anderson, G. Karlström, and H. Wennerström, "On the demonstration of bicontinuous structures in microemulsions", **Colloids and Surfaces**, 1989 38, 205-214.

D. M. Anderson and E. L. Thomas, "Morphology of star diblock copolymers in the strong-segregation limit", **Macromolecules** 1988 21, 3221-3230.

D. M. Anderson and K. Frame, "Teacher access to mentors through professional scientist organizations and the Virginia Science Resource Network", **J. Va. Sci. Ed.**, 2008 2(2):15-24.

Additional book contributions: Geometric Analysis and Computer Graphics, ed. P. Concus, #17 MSRI Series, Springer-Verlag, 1990; Lectures in Minimal Surfaces, J. C. C. Nitsche, Springer-Verlag; NSF Mosaic, "Computer Images in Five Dimensions", ed. W. Kornberg, 1988; Chemical & Engineering News, Aug. 1985; and Islands of Truth, Ivars Pearson, 1992.

PATENTS AND PATENT APPLICATIONS

D.M. Anderson, **U.S. Patent** No. 5,244,799 and associated European Patent #0292145, "Microporous materials."

D.M. Anderson, **U.S. Patent** No. 5,238,613 "Preparation of polymeric hydrogel containing micropores and macropores for use as a cell culture substrate."

C.H. Wick and D.M. Anderson, **U.S. Patent** No. 6,051,189 "System and method for detection, identification and monitoring of submicron particles".

D.M. Anderson, **U.S. Patent** Nos. 6,482,517, 6,638,621, and 6,989,195, and associated international patents, "Coated particle and methods of making and using the same". Continuations of this patent line include U.S. 6,989,195 and U.S. 7,105,229.

D.M. Anderson, **U.S. Patent** No. 6,991,809 "Particles with improved solubilization capacity", and associated international filings.

D.M. Anderson, **EPO Patent** (EP 01998324.6) allowed and currently issuing in European countries, "Solvent Systems for Pharmaceutical Agents", U.S. counterpart pending.

Nine (9) additional patent applications pending, including U.S. 10/889,313 and associated WO application.

TEACHING.

Courses taught.include graduate courses in Biomaterials, Biophysics, and Polymers (University at Buffalo). This included the development of a new graduate Polymers course.
Student supervision includes one PhD and two M.S. students (completed theses/degrees).

Other teaching experience includes Instructor for the Math sections of the MCAT, SAT, GRE and GMAT at Stanley Kaplan Educational Institute (Mpls. office), and tutoring in math, sciences, and engineering both as undergraduate and graduate student at the Univ. of Minnesota, 1974-1981.

INVITED PRESENTATIONS

National / International meetings.

American Physical Society meeting, Pittsburgh, PA, March 1994.
Scientific Conference on Chemical and Biological Defense Research, Aberdeen Proving Ground, MD, Dec. 1994 and November 1995.
Asilomar Conference on Polymers, Monterey, Cal., Feb. 1993.
"Workshop on Ordering in Fluids", Amsterdam, Neth., Sept., 1990.
"Geometry and Interfaces", Aussois, Fr., Sept. 1990.
"Liquids at Interfaces", Les Houches, Fr., June 1989.
European Colloid and Interfaces Society annual meeting, Arcachon, Fr., Sept. 1988 (poster).
"Complex and Supermolecular Fluids", Exxon Corporate Research, July 1985 (poster).
Society of Rheology Meeting, Knoxville, Nov. 1984.
Society of Pure and Applied Mathematics, Seattle, July 1984.
Microscopy Society of America annual meeting, Cincinnati, August 1993.
Oak Ridge Conference of the American Association of Clinical Chemists "Pushing the Envelope II", April 2005.

Dozens of industrial labs.

Defense Department. Invited lectures at the Naval Research Laboratories and Army Edgewood Arsenal (1994 and 1995 Annual Conferences on Chemical & Biological Defense Research), in addition to the DoD-sponsored Asilomar and Chem/Biol Defense conferences cited above. Also site-visited for a block grant proposal I P.I.'d, with a 5-year budget of $2.1 million, selected as one of the top 3 among 227 competitive proposals.

Universities. University of Buffalo, Departments of Biophysics, Oral Biology, Chemical Engineering, Chemistry, and the Roswell Park Cancer Institute; also the Western New York Science Forum and the NSF co-sponsored "Nanobiology at Interfaces" symposium. Also at Princeton (Physics Dept., at the invitation of Sol Gruner, and Chemical Engineering / Princeton Materials Institute, at the invitation of Bob Prud'Homme); Cornell (Physics, Stanislas Leibler); MIT (Materials Science and Eng., Edwin Thomas); James Madison University (Biotechnology Association); Umea Univ (Biochemistry, Goran Lindblom); U. Lund (Chemical Technology, Bertil Tornell); U. Arizona (Biochemistry, David O'Brien); U. Wash. Seattle (Chem. Eng., Eric Kaler); U. Michigan (Materials Science, David Martin); McMasters (Biochemistry, Materials Research Center); Medical College of Virginia (Division of Neurosurgery, Timothy VanMeter).

VOLUNTEER WORK

2005-present: Chair, Government Relations Committee, Virginia Section of the American Chemical Society. Working to coordinate ACS member involvement in with governing bodies in science education, legislation and budgeting, as well as in student mentoring, equipment procurement for middle and high schools, and changes in science fair rubrics.

Authored an invited paper for the Journal of Virginia Science Education (JVSE) entitled "Teacher Access to Mentors through Professional Scientist Organizations: The Virginia Science Resource Network" (in press).

Judge at Virginia State Science & Engineering Fair, Metro Richmond Science Fair, Virginia Junior Academy of Science fair; presentations at Career Days, SMV Lunch Series, Chemistry Week events, mentor science projects, etc

Advisor to Hanover County Science Curriculum Committee. Advise particularly in the area of science projects, instrumentation, and fairs, as well as textbook and curriculum choices.

Attachment 2 to Declaration of David M. Anderson

1. (See Plate 1) Four of the five Embodiments of the Japanese patent were produced as set forth in that disclosure, without any additional processes or procedures, such as homogenization, introduced. These were shaken by hand and examined very shortly thereafter. The vials are approximately 17.5 mm outer diameter. A droplet was also drawn off in each case and examined in a DIC (differential interference contrast) microscope, with a 100x objective, 6.3x eyepiece, and 0.8x lens in between.

It is clear from each of the micrographs that clumps in excess of 30 microns in diameter are present. While this alone would make the formulation unsafe for intravenous injection, the vial pictures show matter at the bottom of the vials that is on the order of ¼ millimeter, or 250 microns. After 5 minutes of sitting, most of the dantrolene has in fact settled to the bottom half of the liquid, indicating a fast sedimentation and thus large particle size (particularly since the density of dantrolene sodium is only slightly greater than that of water).

2. (See Plate 2) Two of the same Embodiments (#1 and #5) were made in larger batches so that the additional process step of homogenization, not identified in the Japanese patent, could be attempted. The homogenization applied, 15 minutes at 10,000 RPM, was exactly the same, and on the same homogenizer, as performed during the production of the nanocrystal dispersion produced by the inventors. As in the previous experiment, samples were analyzed shortly after hand shaking. In the photographs of the vials, a ruler marking off 20 millimeters is superposed on the photos. The vials again are approximately 17.5 mm outer diameter. As above, a droplet was also drawn off in each case and examined in a DIC microscope. In these micrographs, a 20-micron (not to be confused with 20 millimeter) bar is superposed on the micrograph.

Embodiment #1 settled quickly after shaking—and mostly to the top of the liquid, underscoring the near-neutral density of the particles—and in the microscope showed huge particles, greater than 50 microns in size. Embodiment #5 did not completely settle after 5 minutes, but microscopy showed a predominance of long needle-shaped crystals, typically about 40 microns in length, many longer, making this unsafe for i.v. injection.

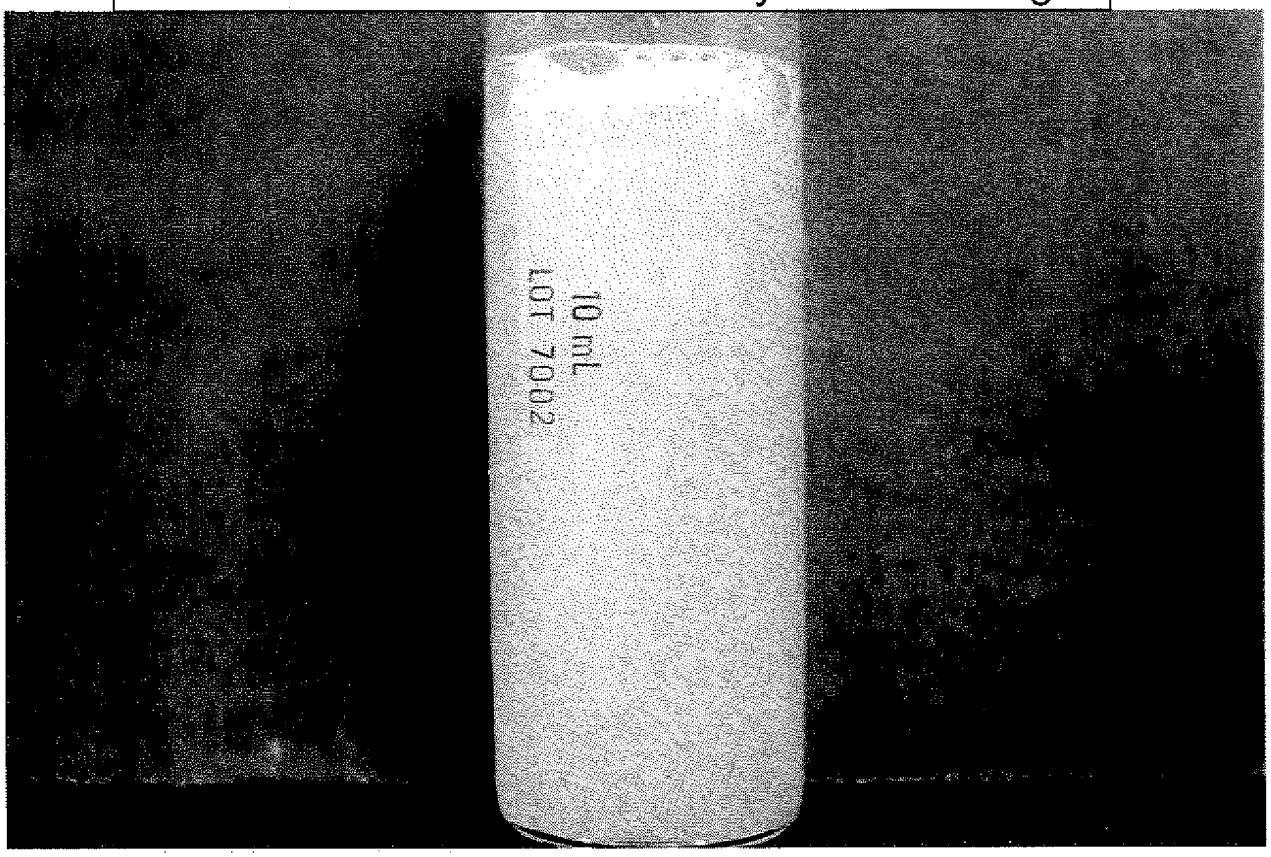
3. (See Plate 3) In another attempt, the same homogenization protocol which is applied to the Instant Invention was applied to Embodiment #5 of the Japanese patent. Even after 90 minutes of homogenization using this Embodiment #5 formulation, a high content of particles at or above the 8 micron particle size are present, and very little if any particle size reduction has occurred. Indeed, homogenization was stopped at 90 minutes because it appeared that continued homogenization was actually increasing the particle size, probably by inducing clumping. While particles of this size and morphology are safe for oral administration, they are unsafe for i.v. administration.

Plate 1

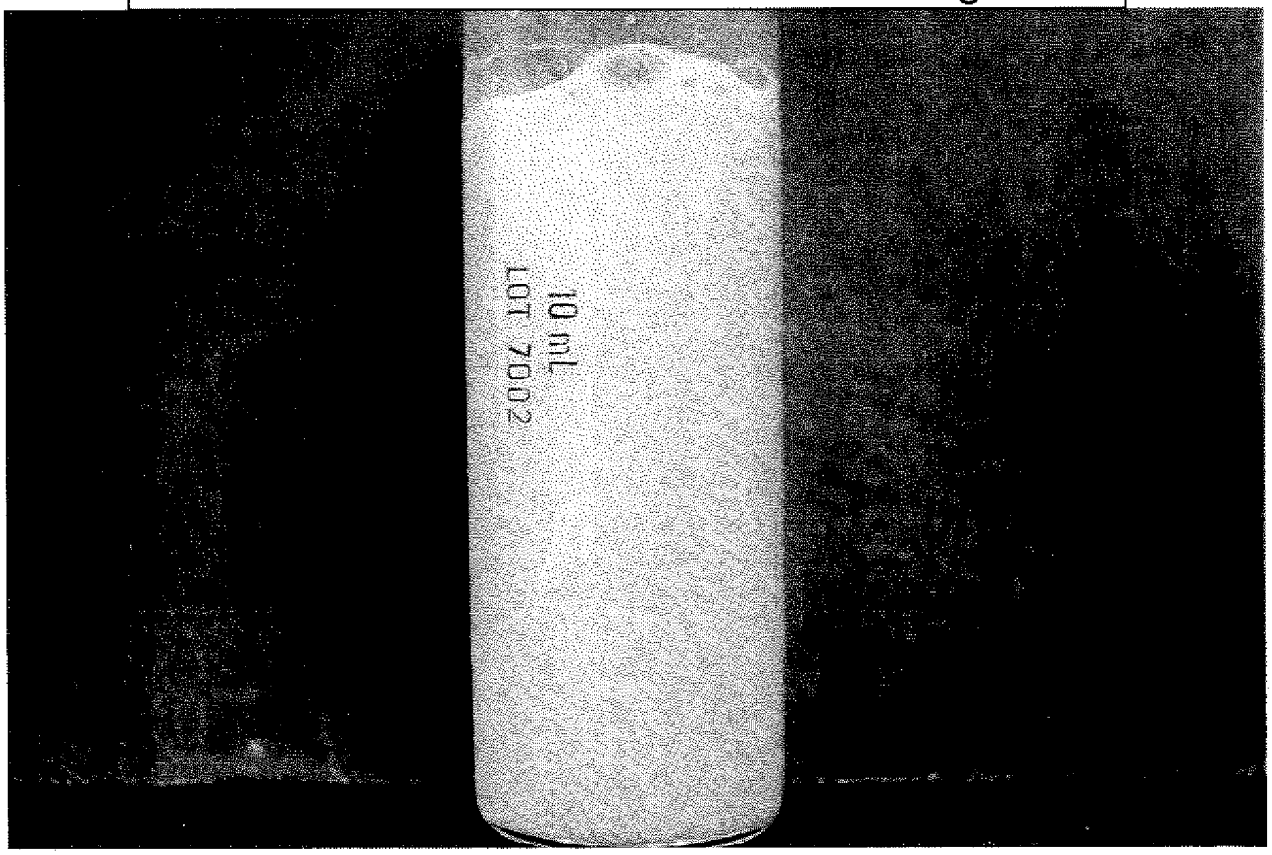
Attachment 2 to Declaration of David M. Anderson

- Embodiment 1:
  - 2.5g sucrose, 85mg sodium citrate, 20mg methyl paraben, 50mg Dantrolene, 5.35g water
- Embodiment 2:
  - 2.5g sucrose, 0.113g potassium tartrate, 20mg methyl paraben, 50mg Dantrolene, 7.3g water
- Embodiment 4:
  - 2.5g sucrose, 58mg sodium chloride, 20mg methyl paraben, 50mg Dantrolene, 7.4g water
- Embodiment 5:
  - 50mg Dantrolene, 85mg sodium citrate, 20mg methyl paraben, 2.33g sucrose, 15mg hydroxypropyl cellulose, 7.5g water

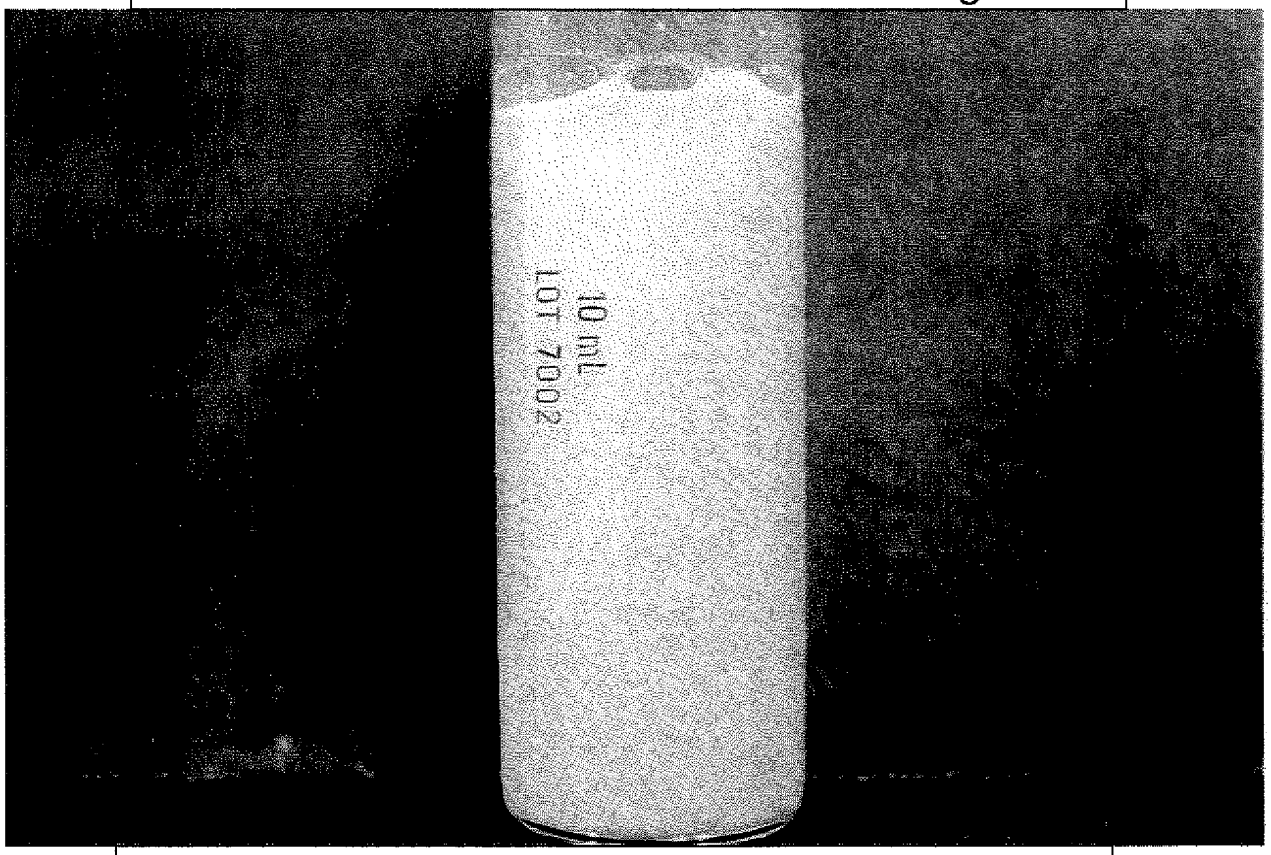
Embodiment 1 – Immediately after shaking



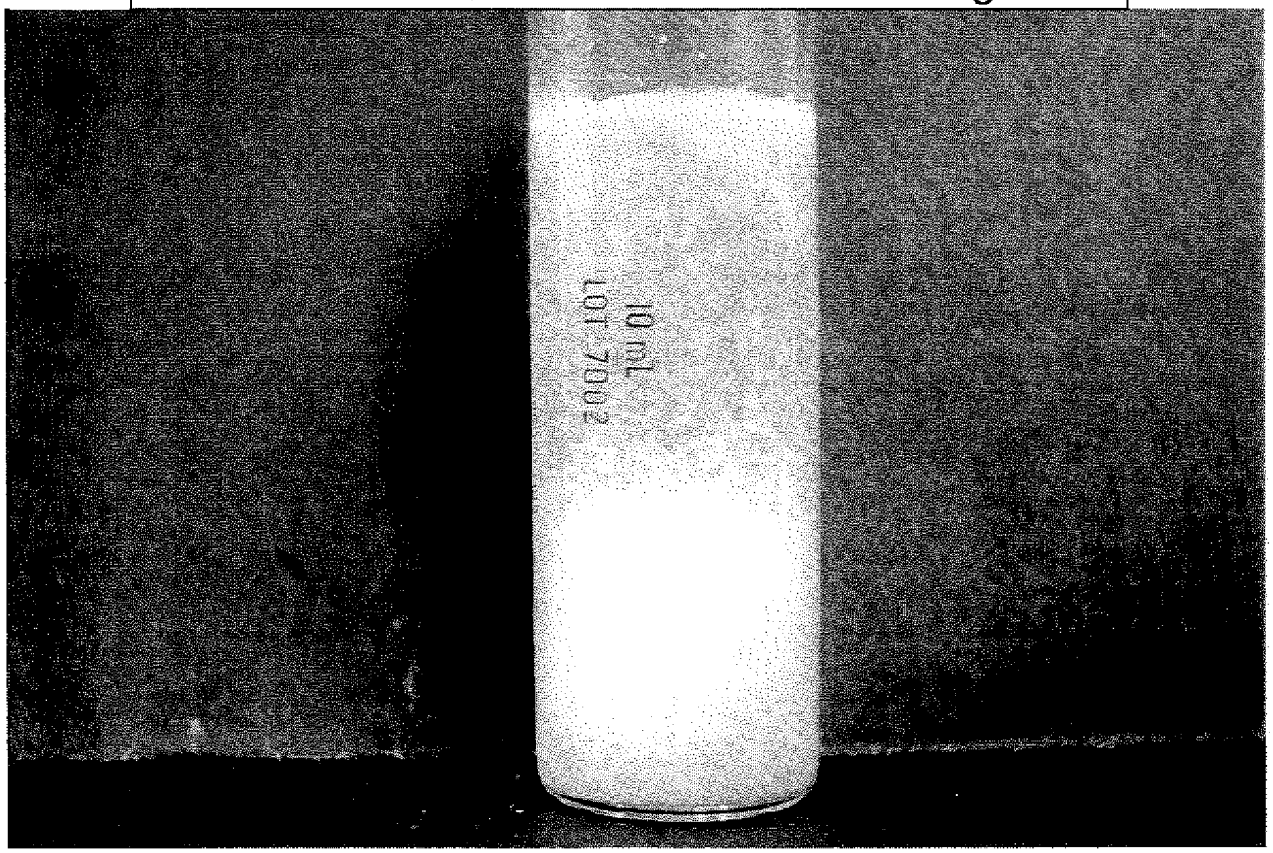
Embodiment 1 – 15s after shaking



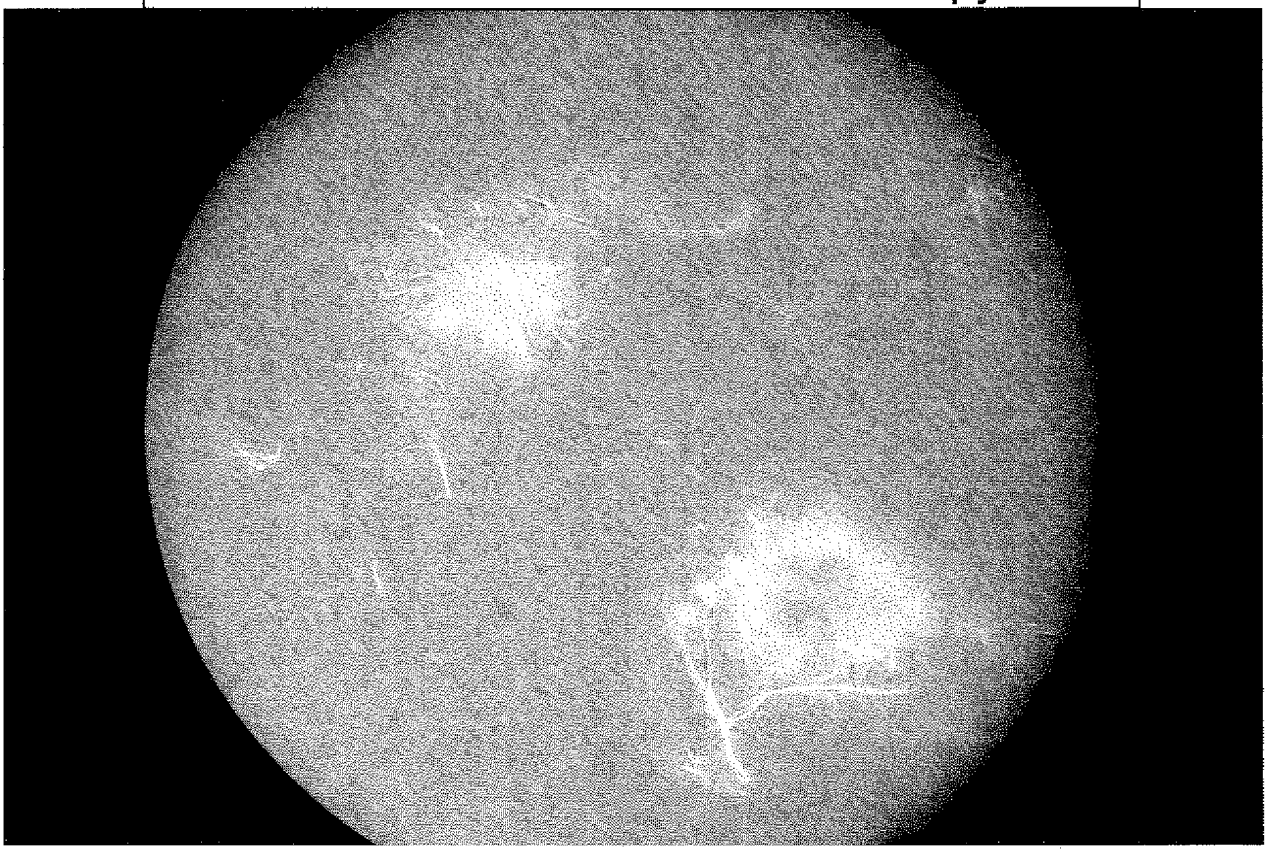
Embodiment 1 – 30s after shaking

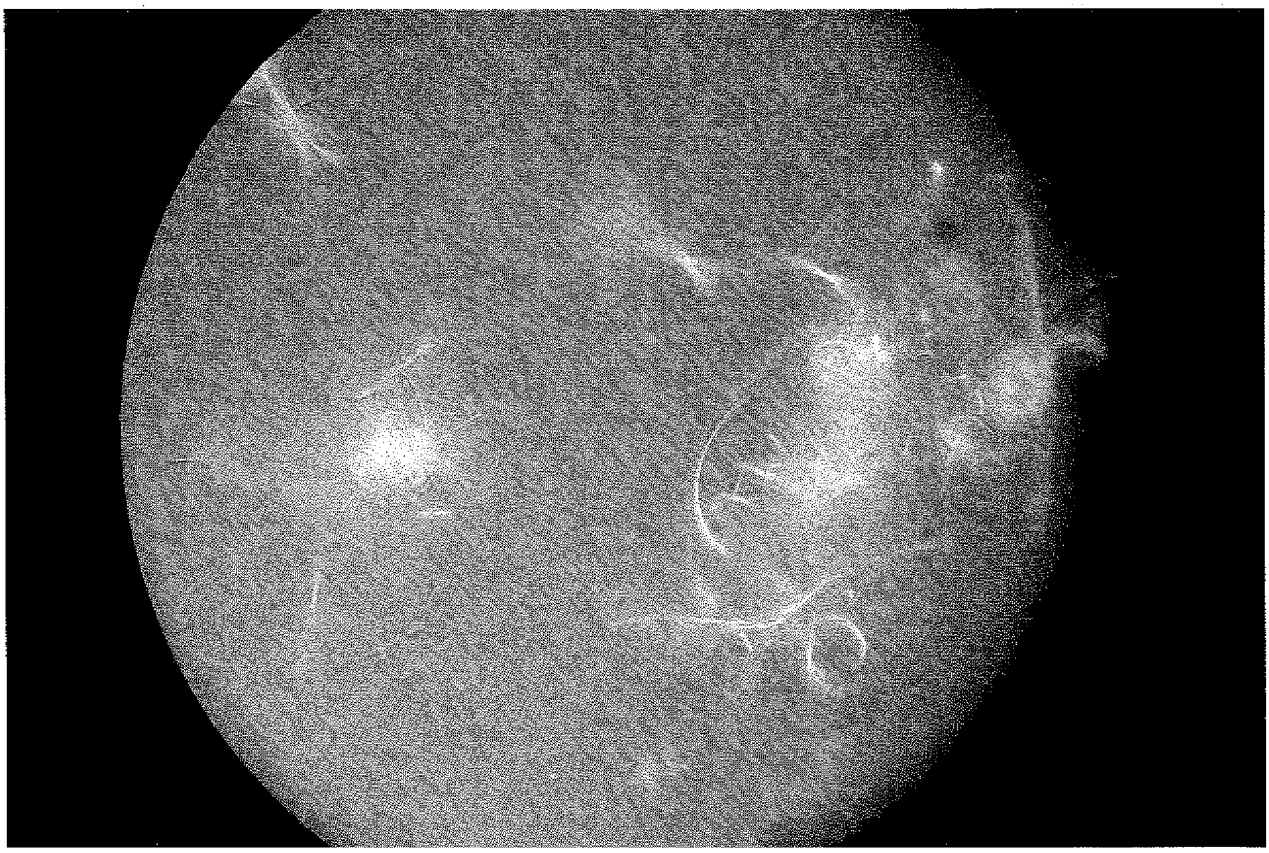


Embodiment 1 – 5min after shaking

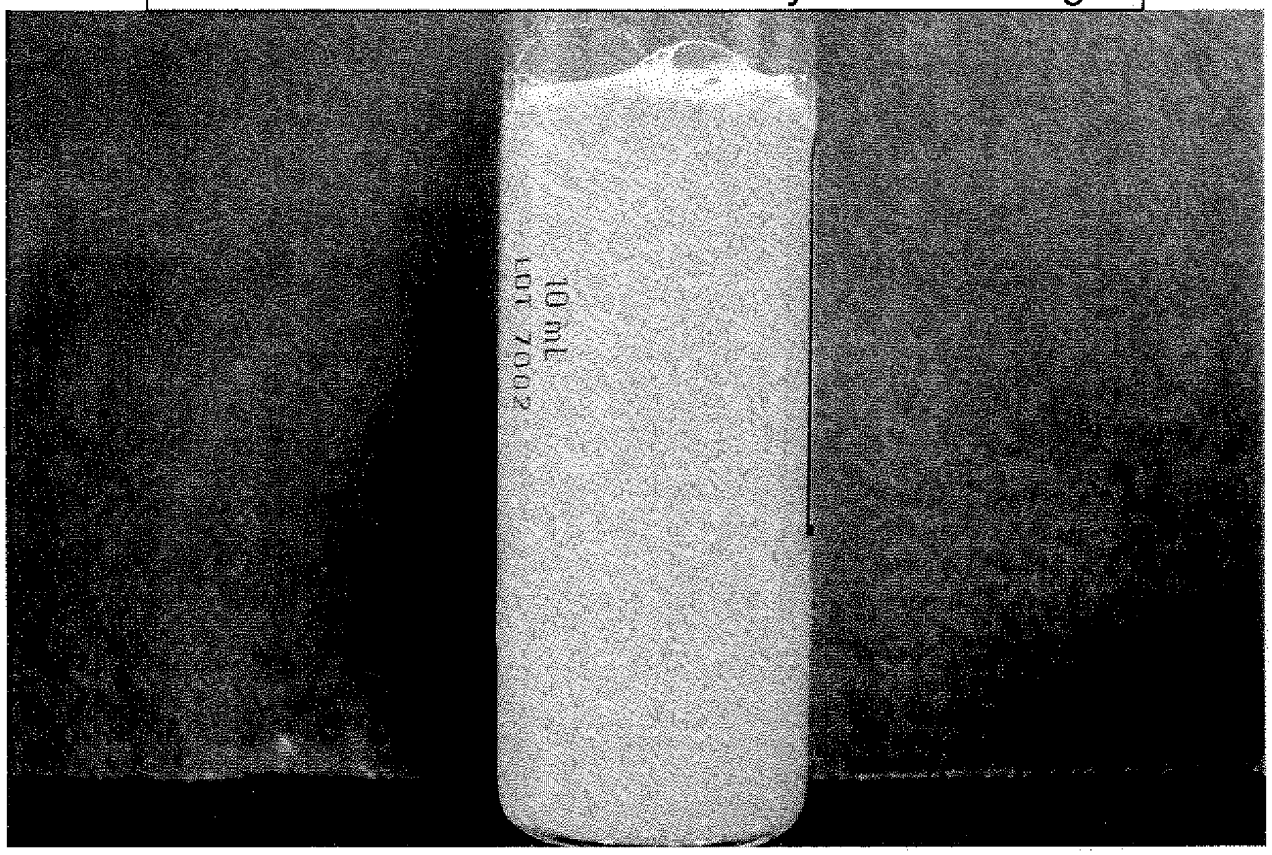


Embodiment 1 – Initial Microscopy

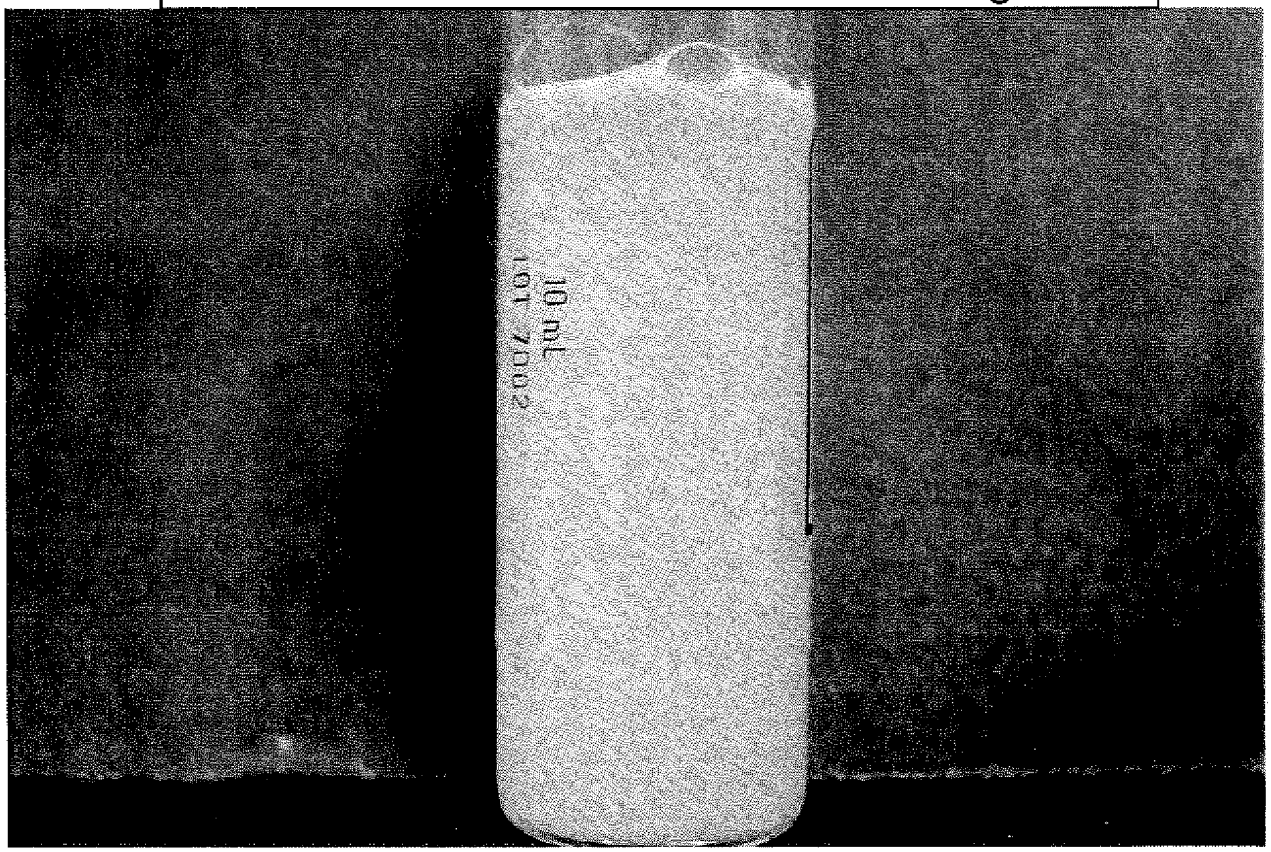




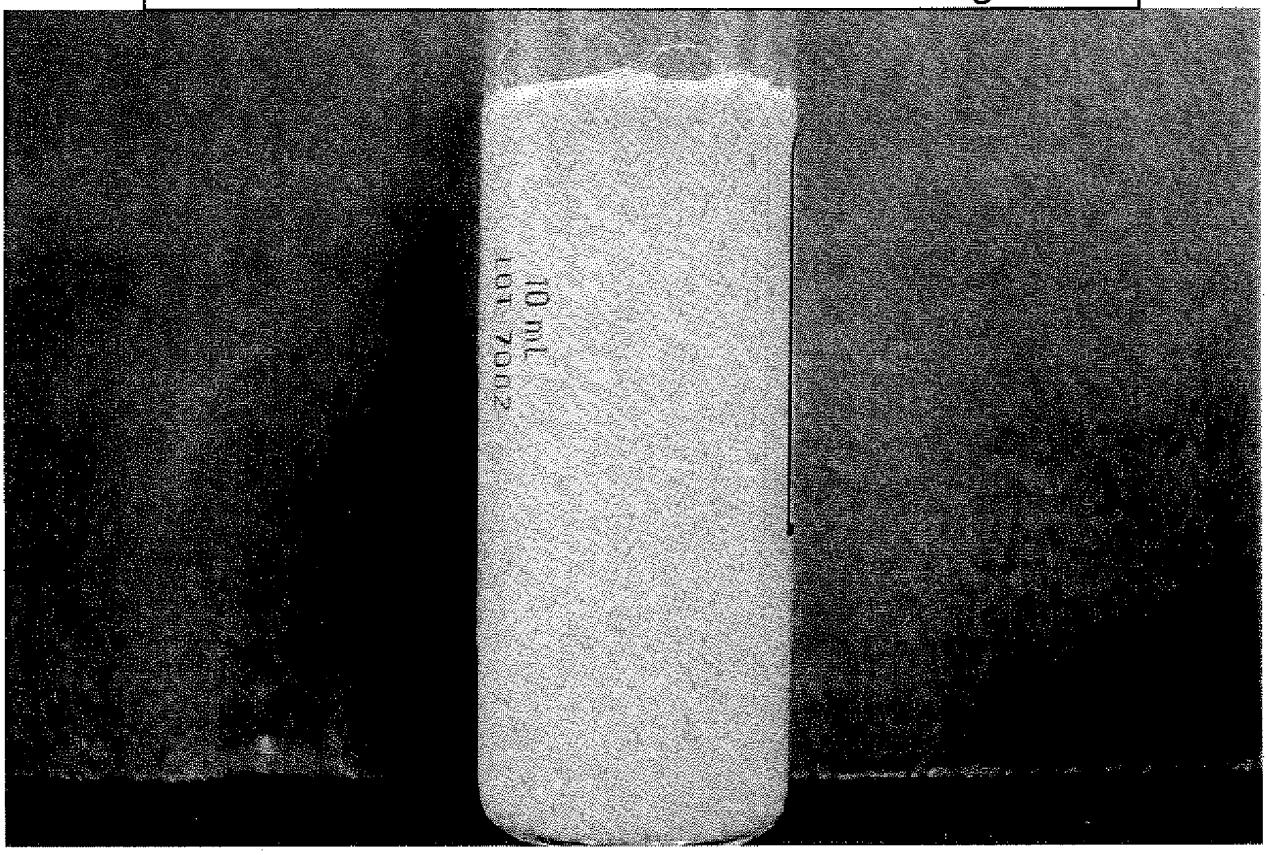
Embodiment 2 – Immediately after shaking



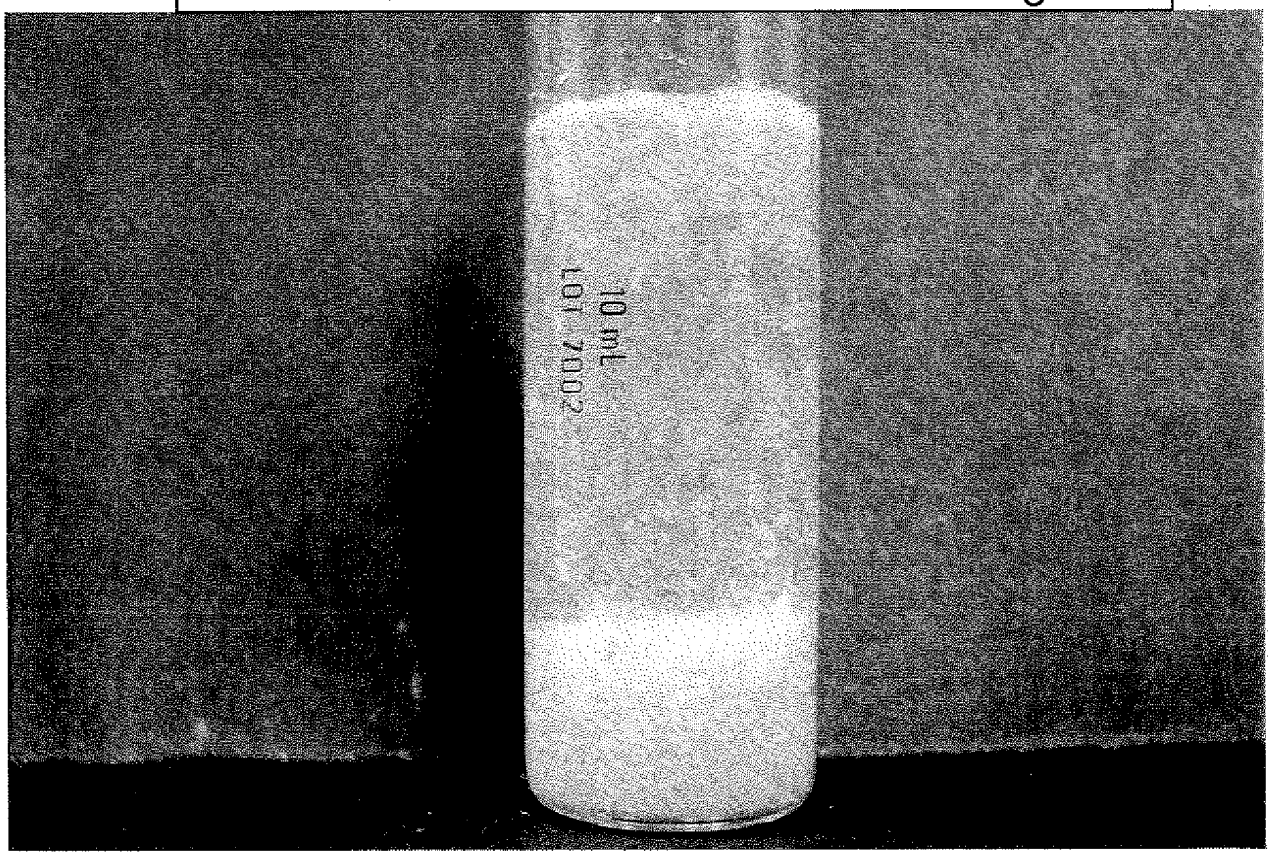
Embodiment 2 – 15s after shaking



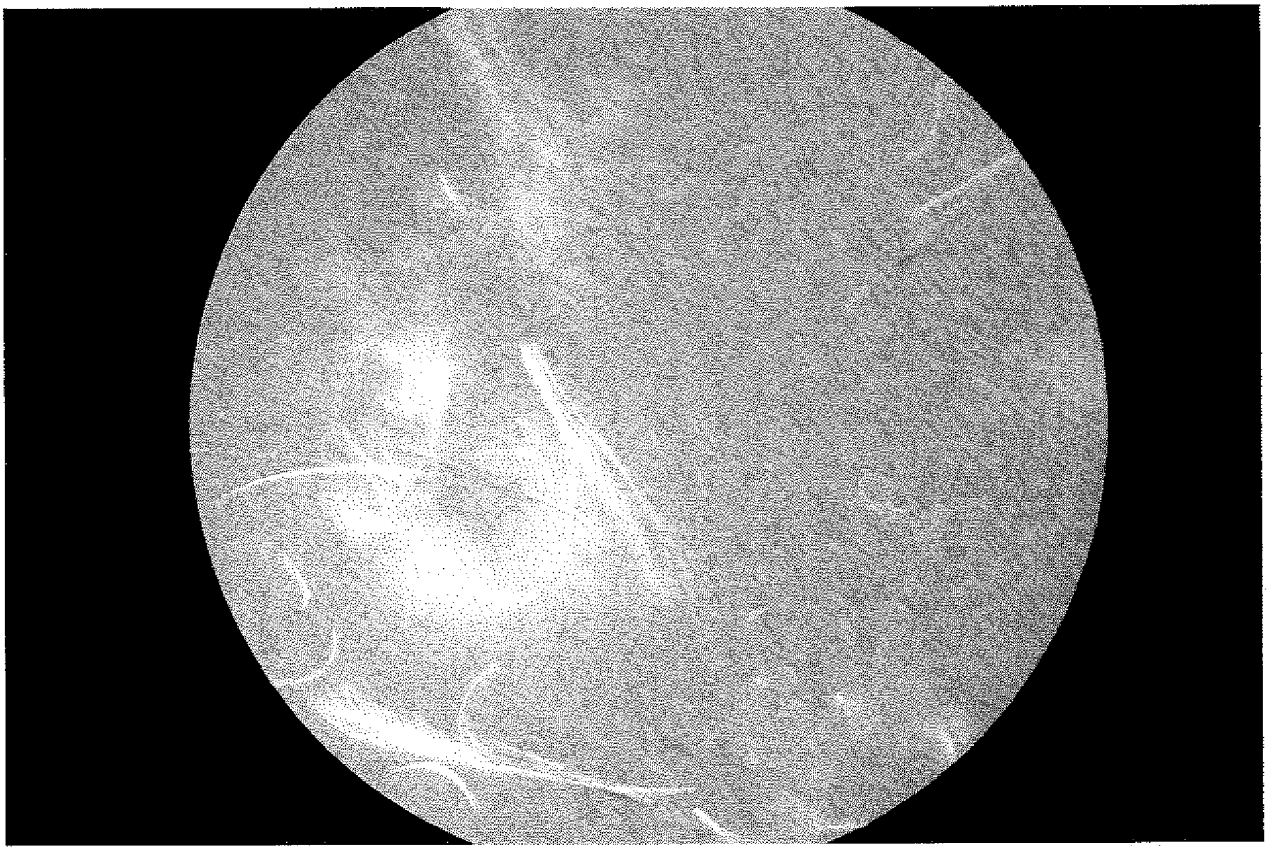
Embodiment 2 – 30s after shaking

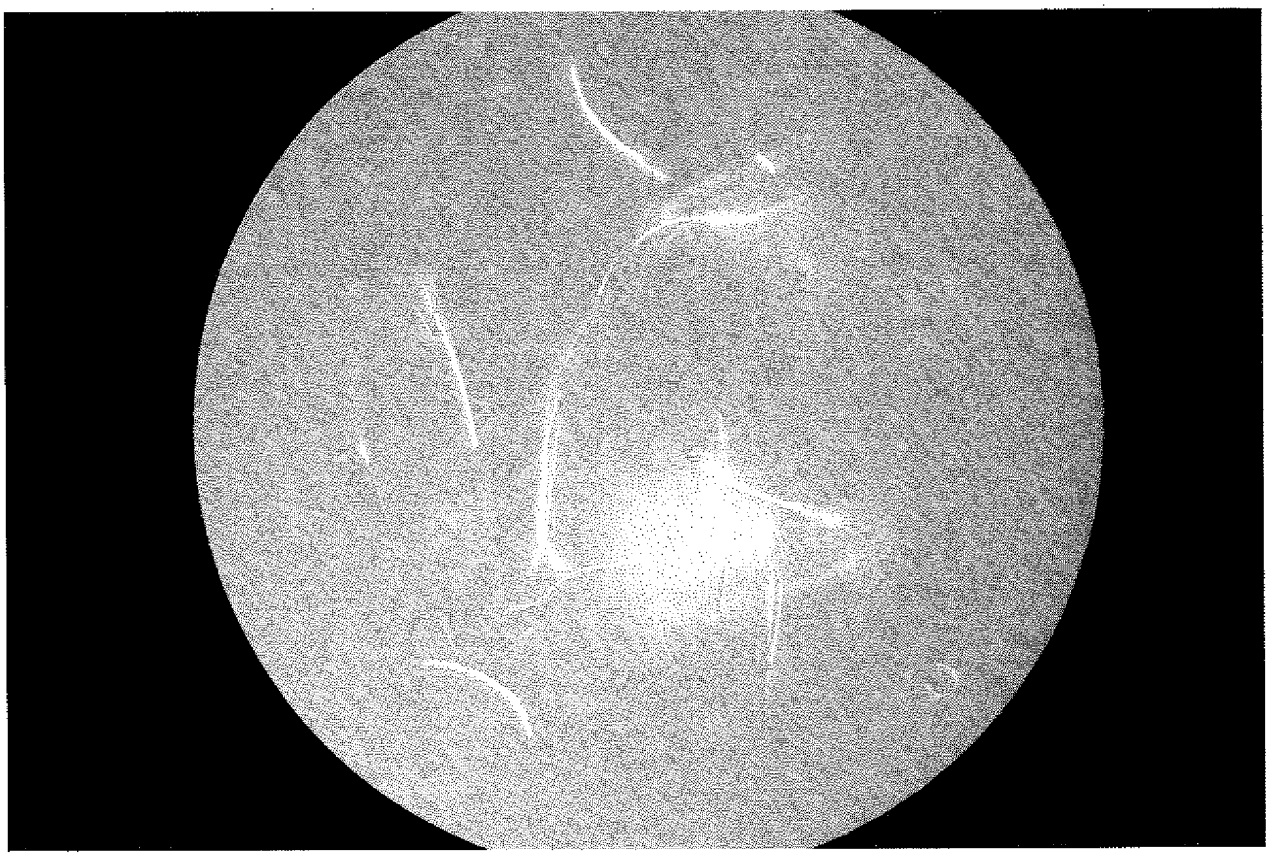


Embodiment 2 – 5 min after shaking

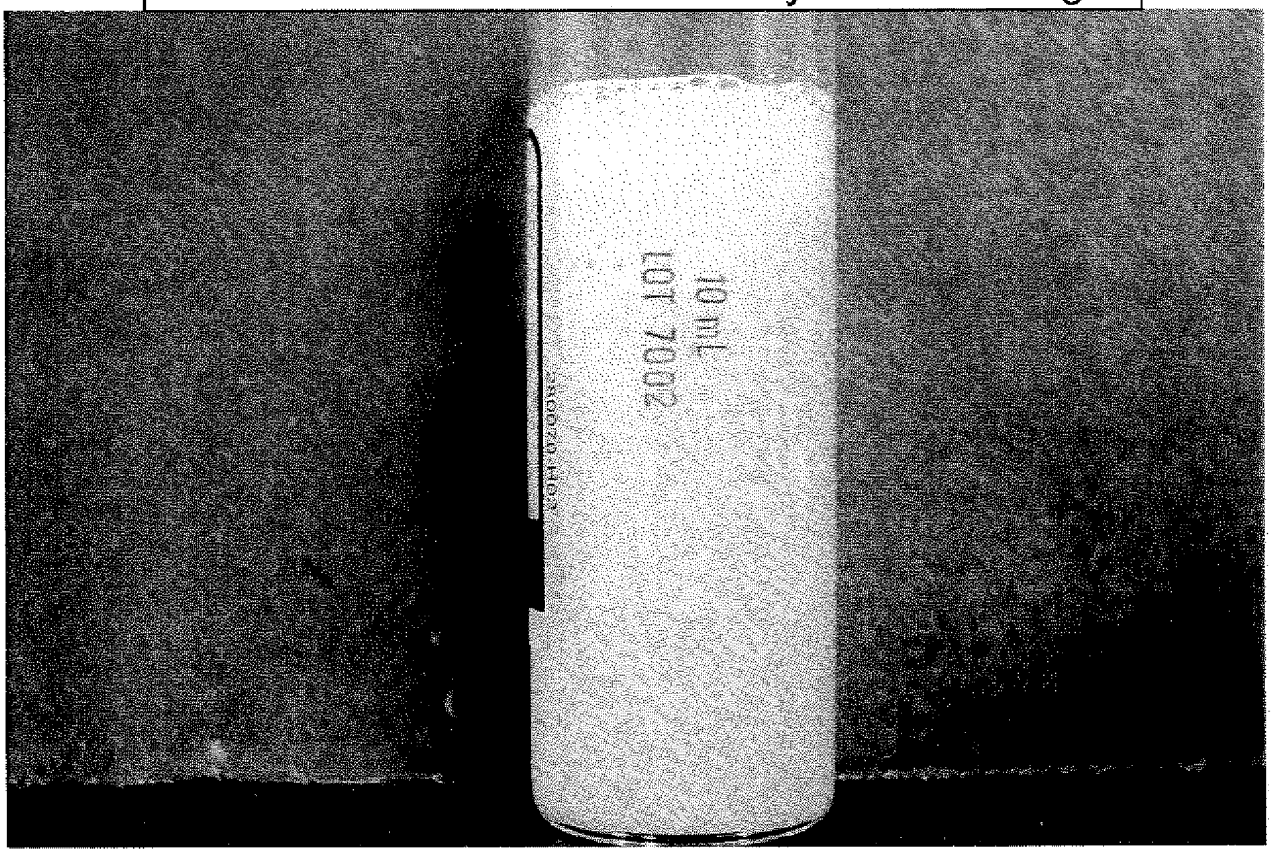


Embodiment 2 – Initial Microscopy

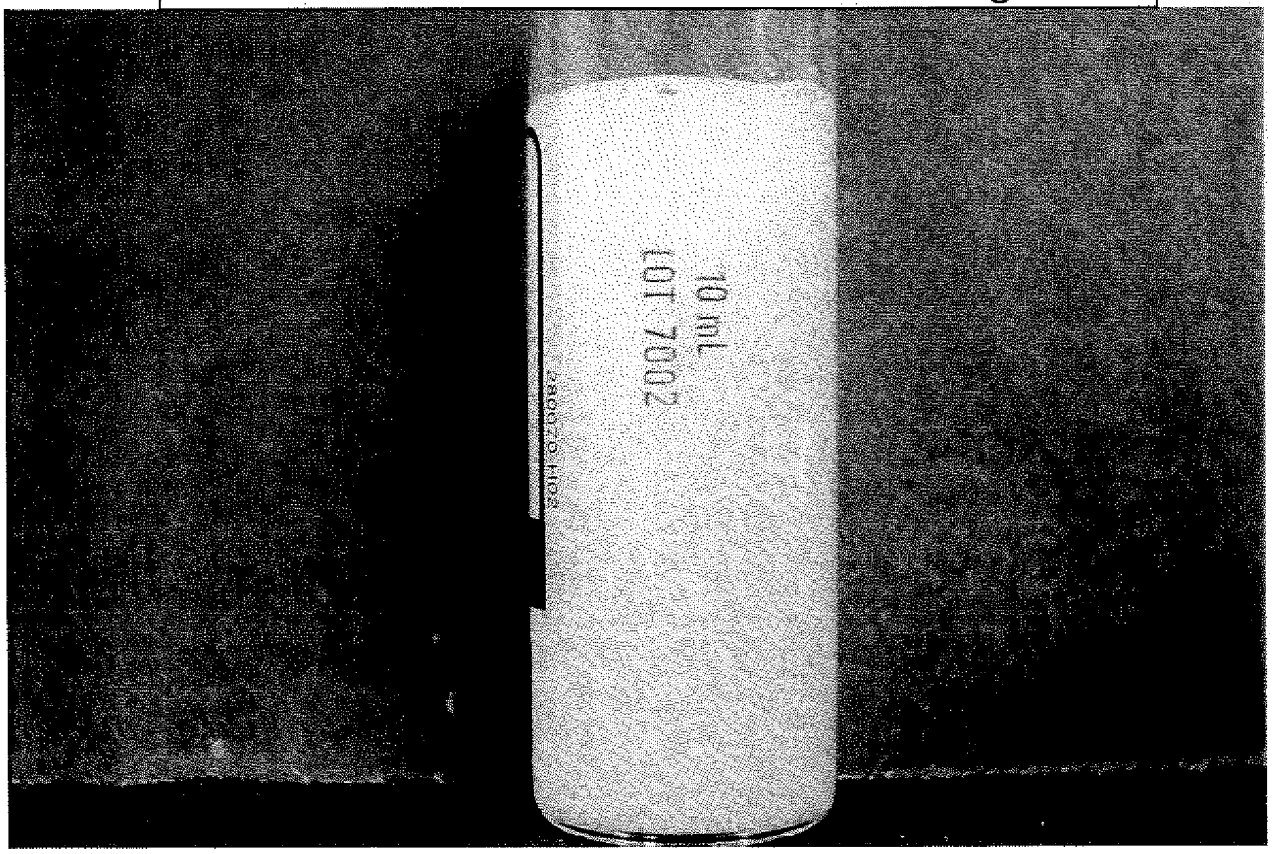




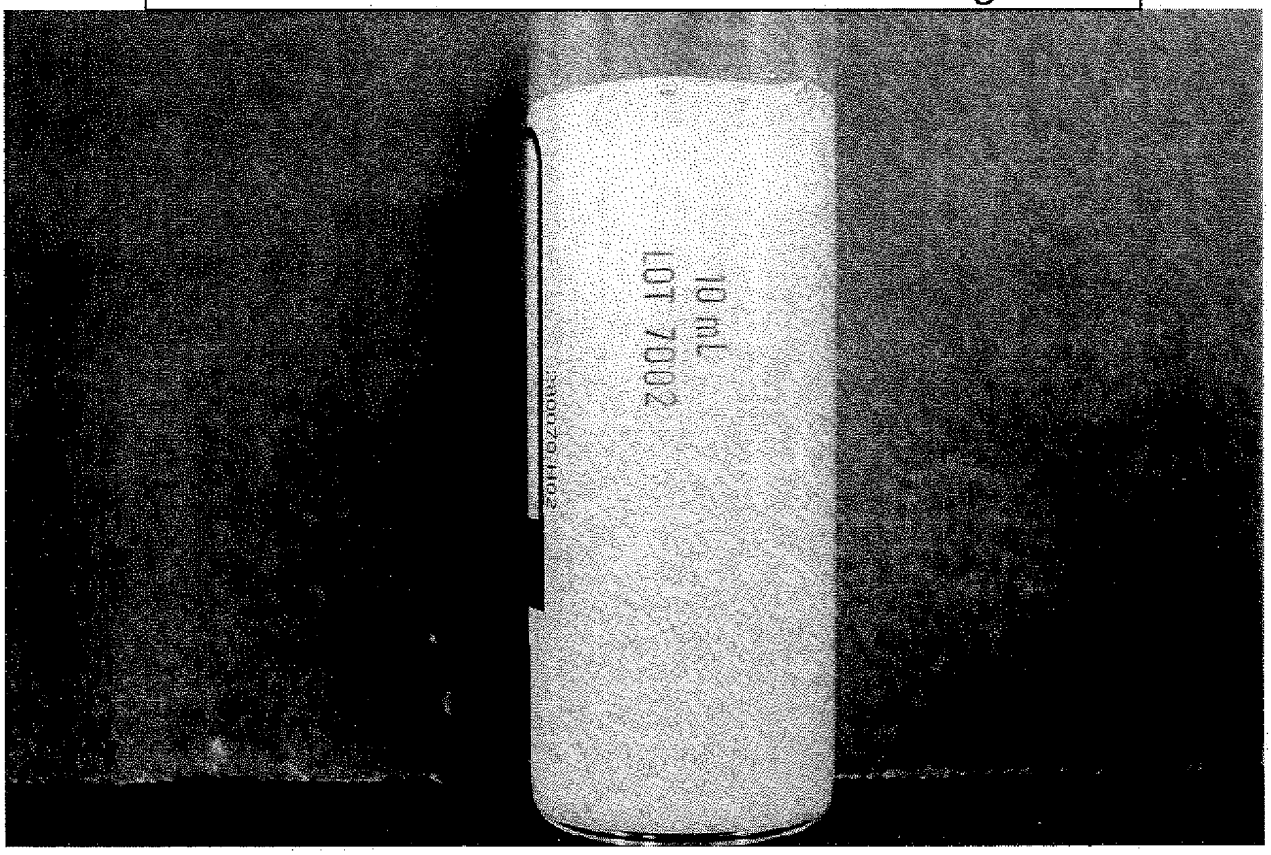
Embodiment 4 – Immediately after shaking



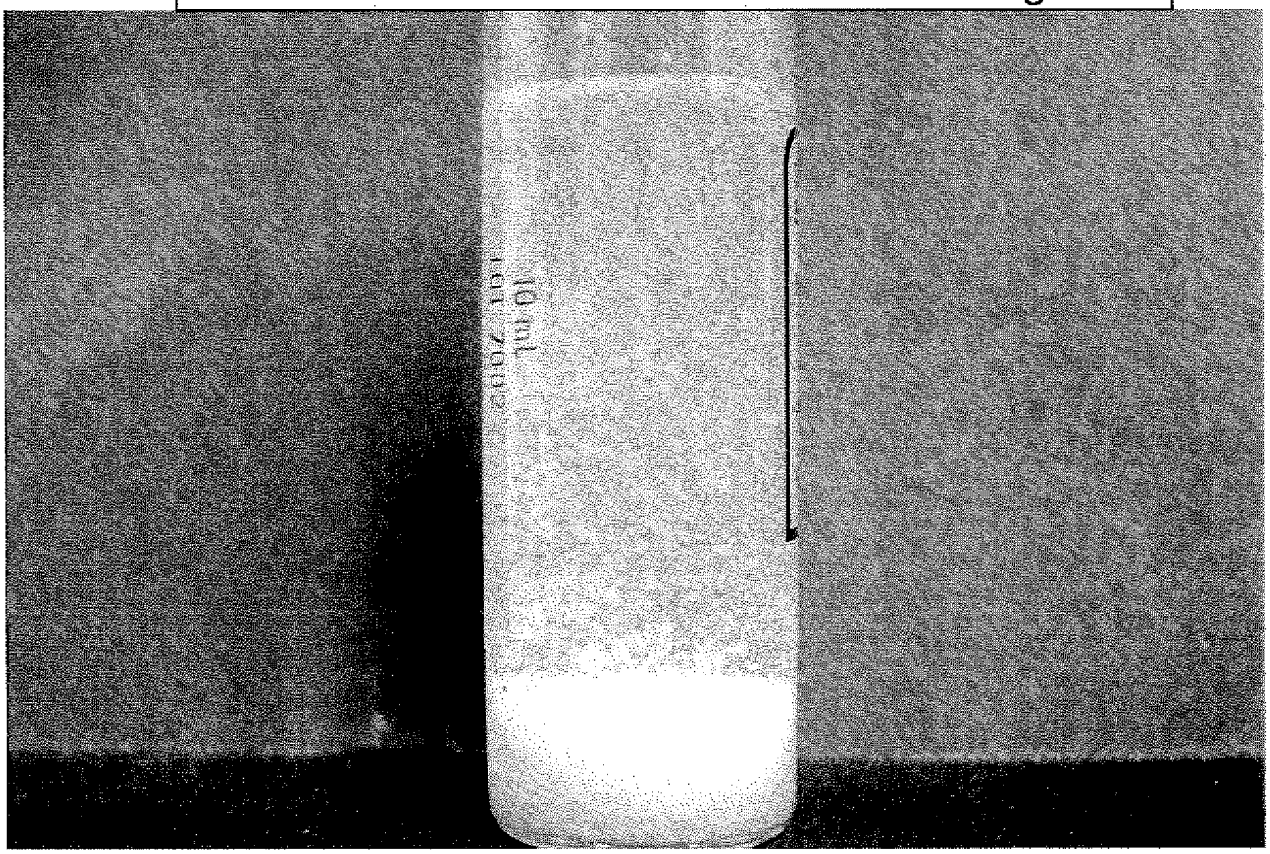
Embodiment 4 – 15s after shaking



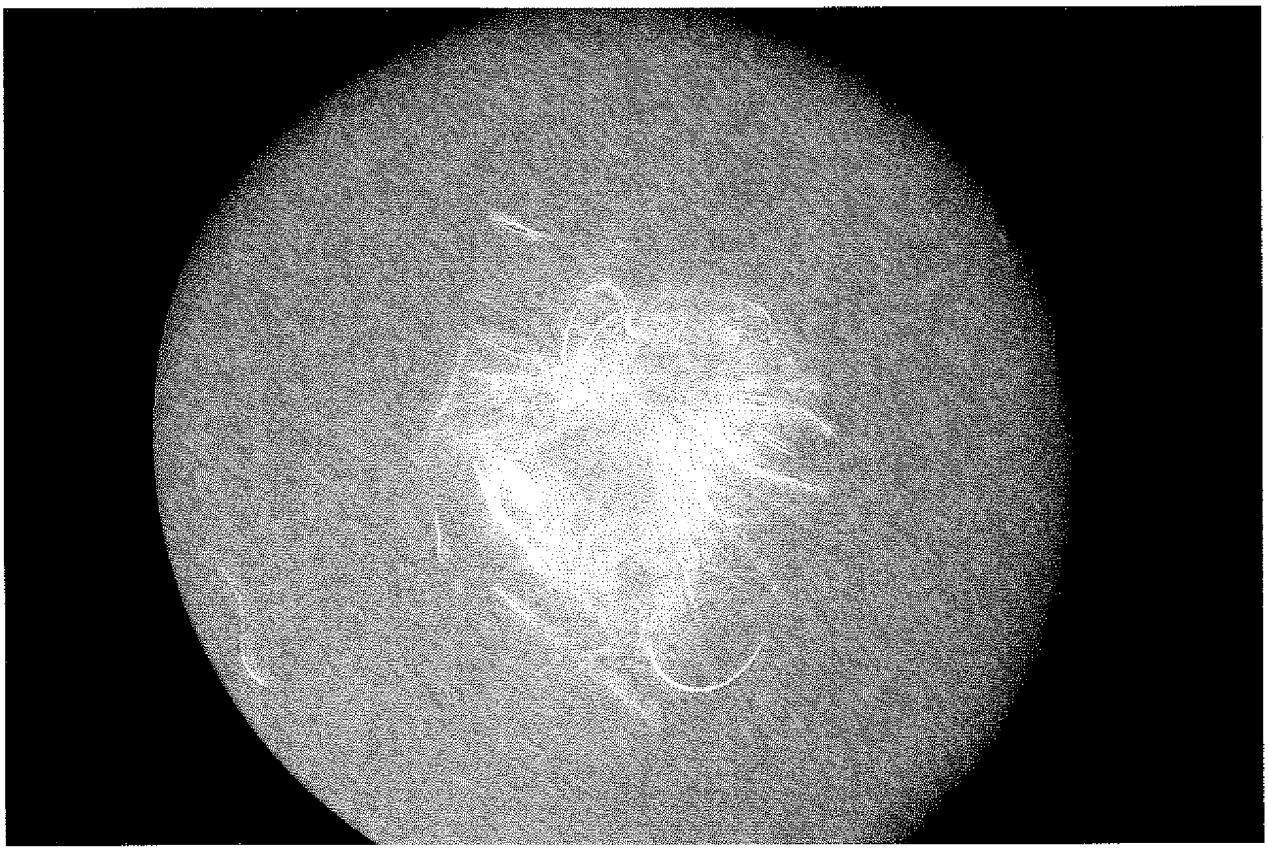
Embodiment 4 – 30s after shaking

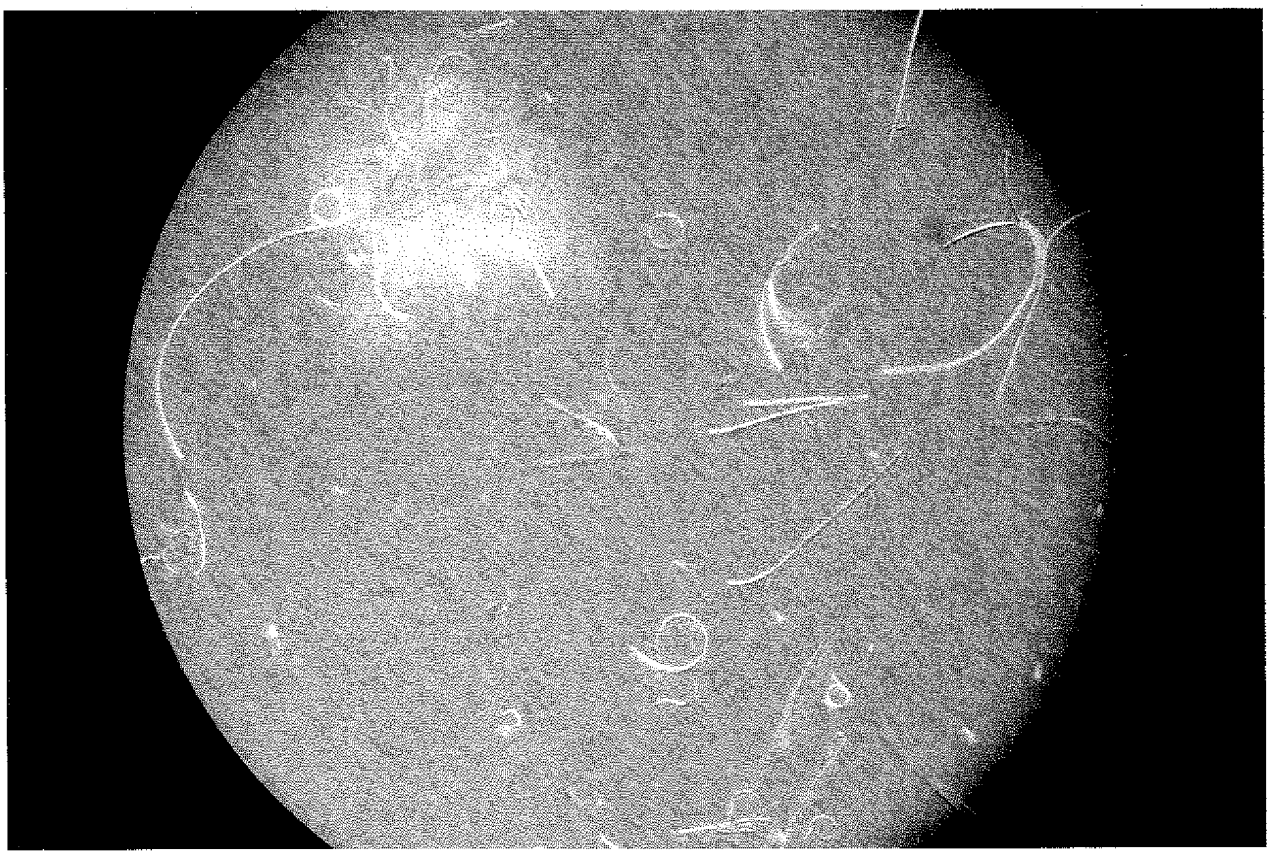


Embodiment 4 – 5 min after shaking

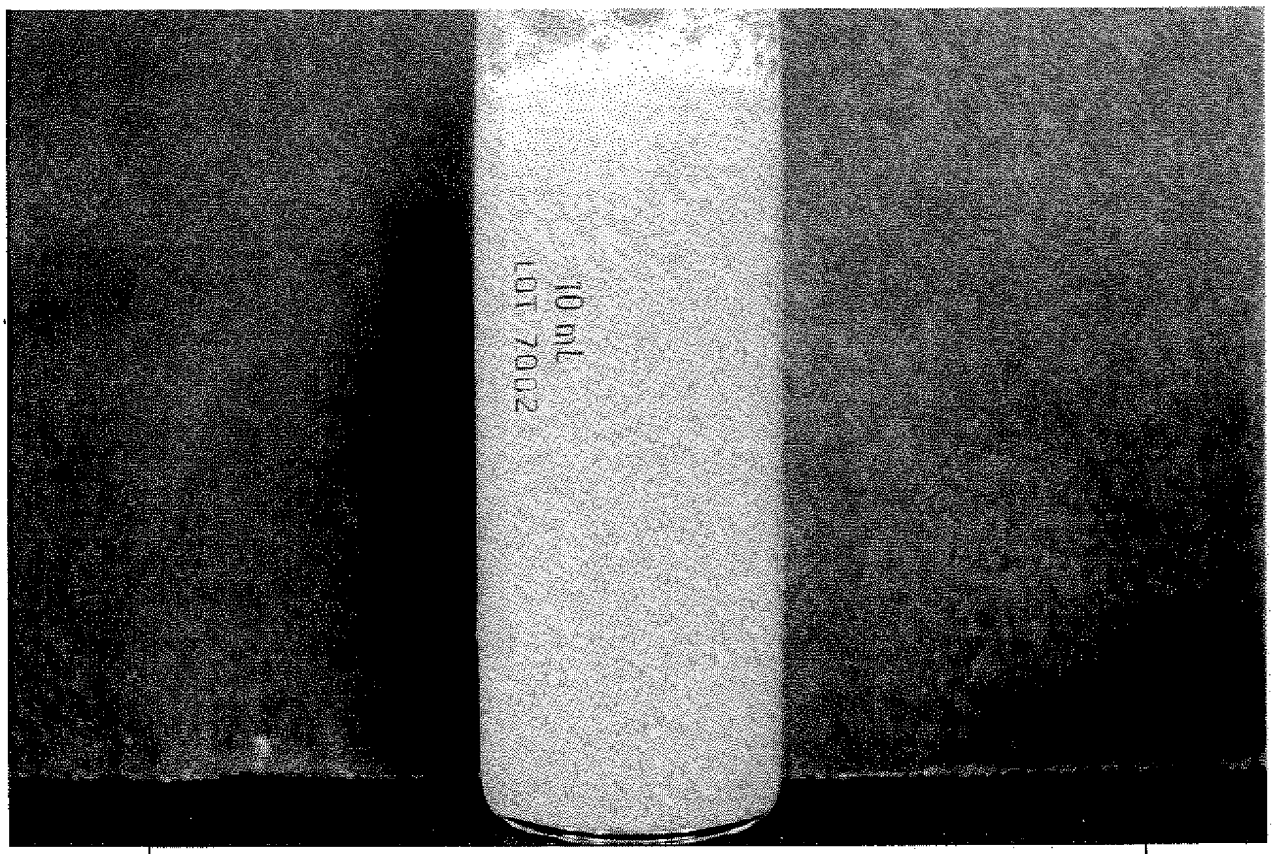


Embodiment 4 – Initial Microscopy

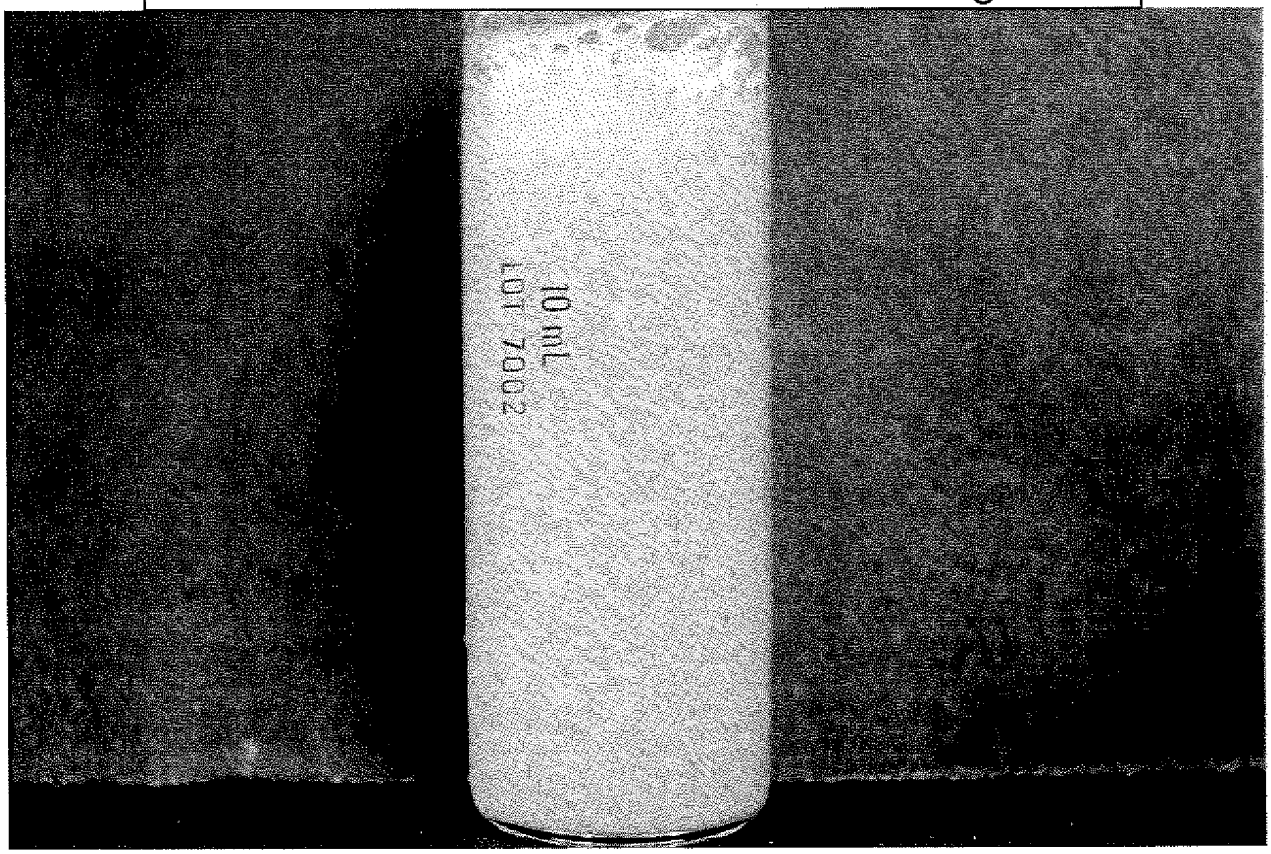




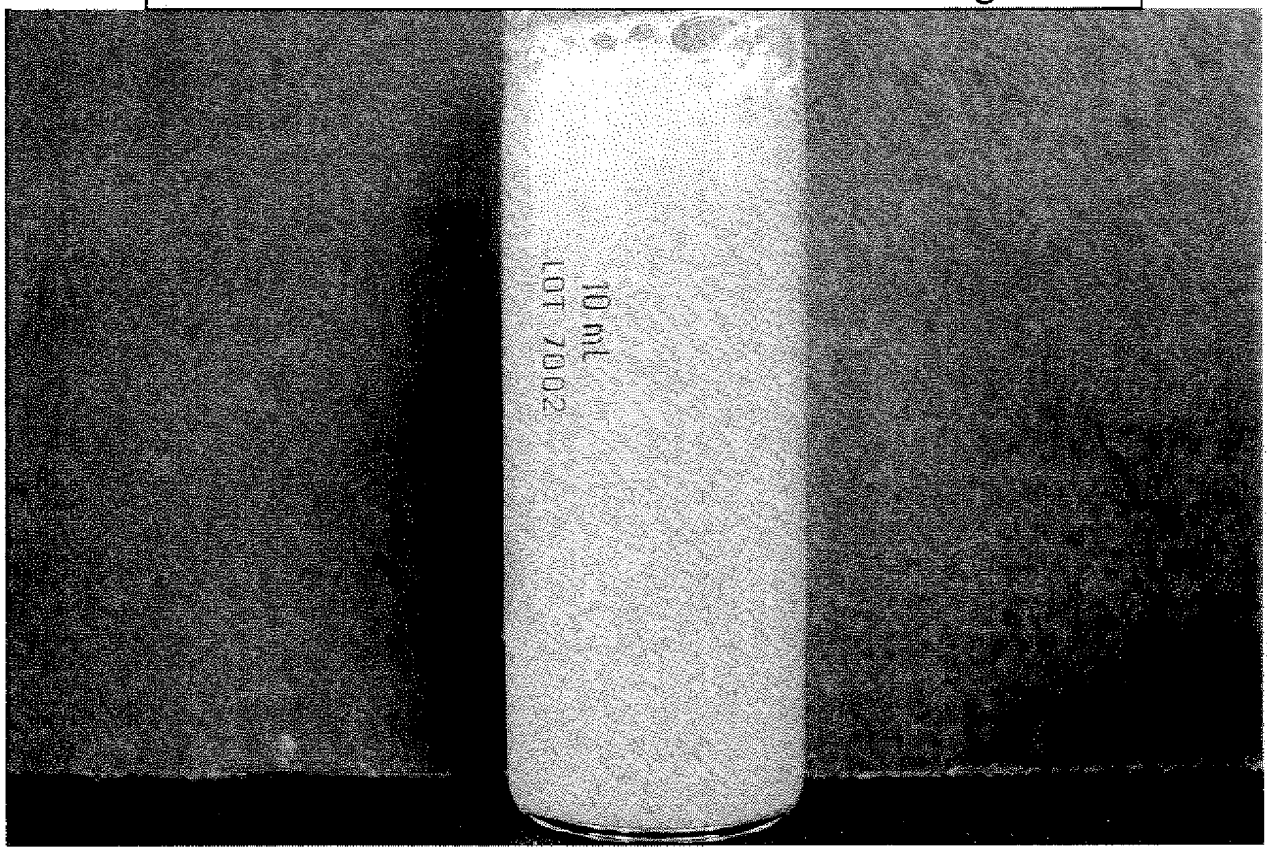
Embodiment 5 – Immediately after shaking



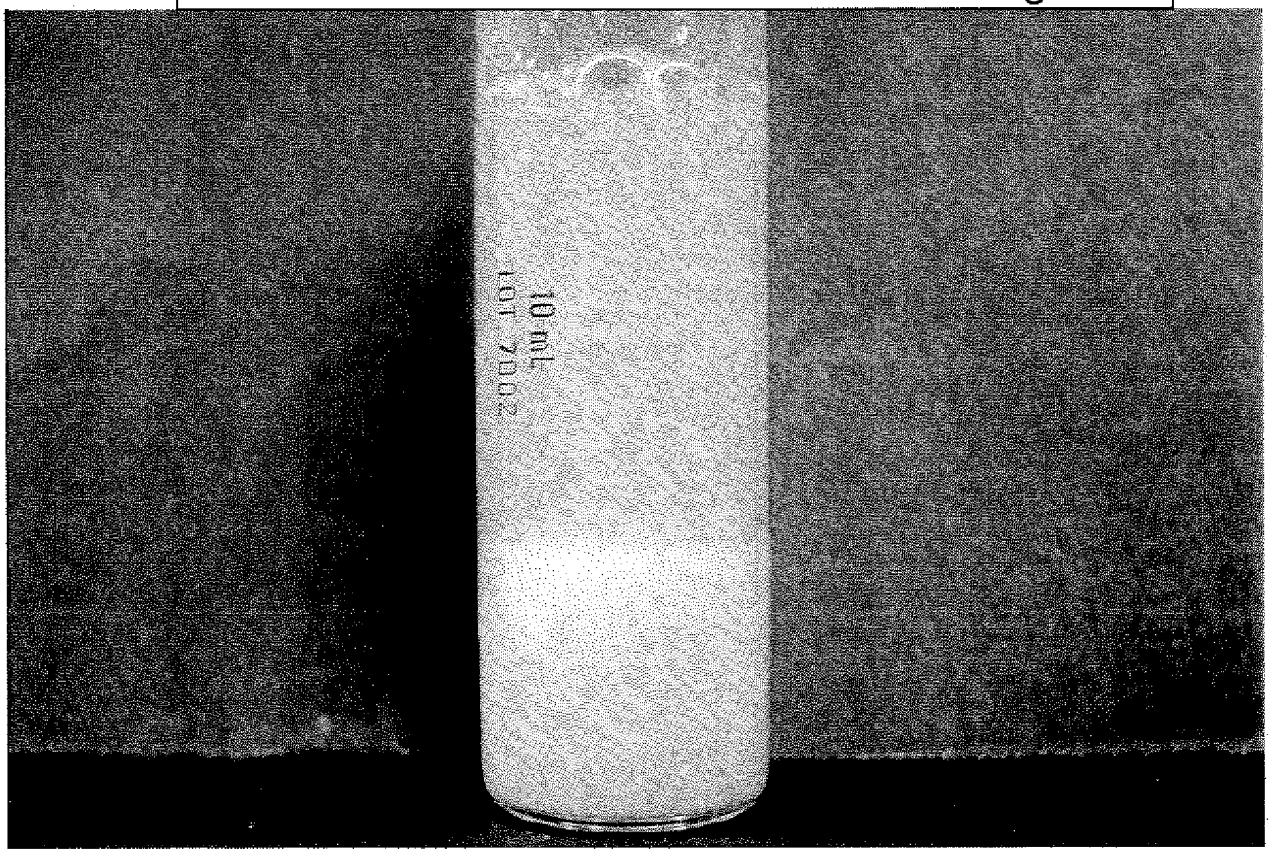
Embodiment 5 – 15s after shaking



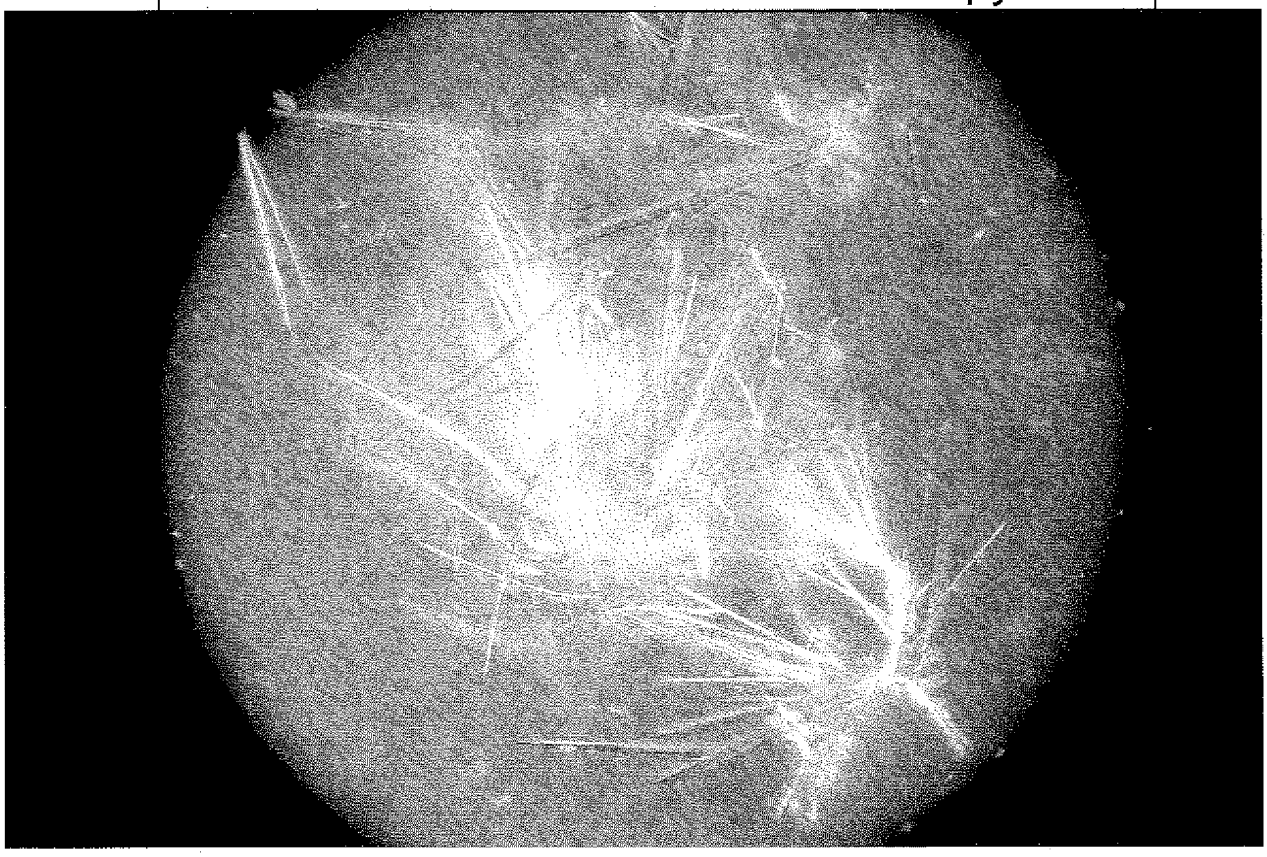
Embodiment 5 – 30s after shaking



Embodiment 5 – 5 min after shaking



Embodiment 5 – Initial Microscopy



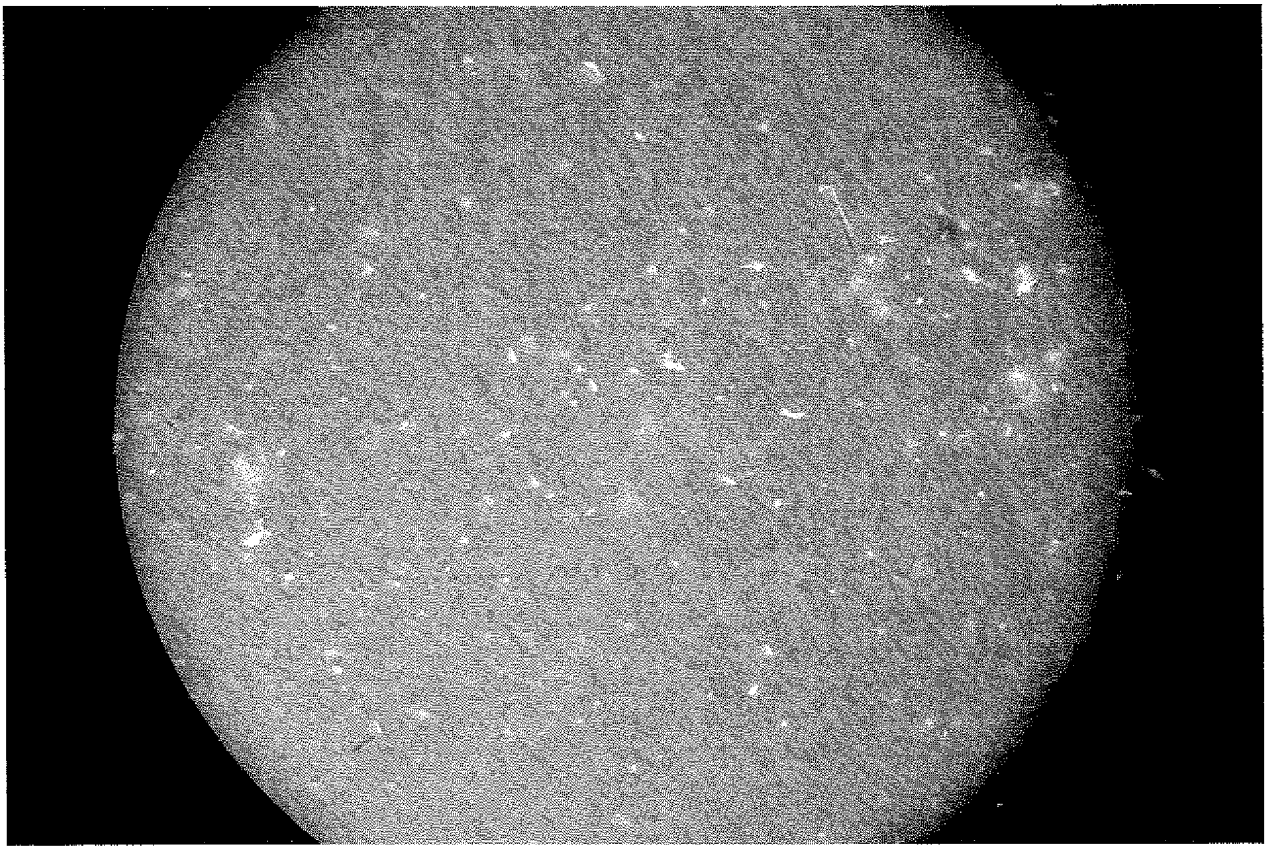
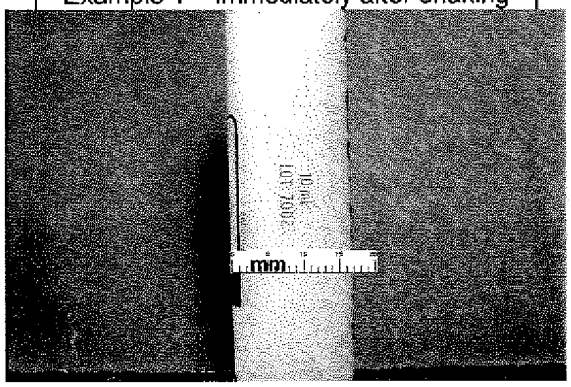


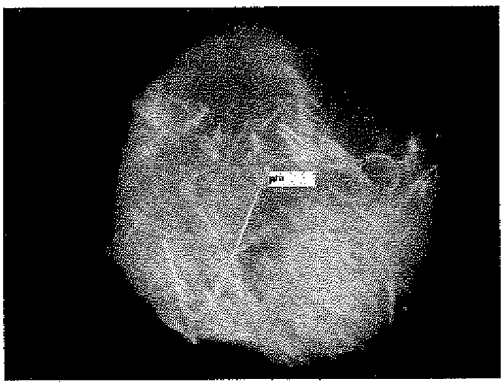
Plate 2

Attachment 2 to Declaration of David M. Anderson

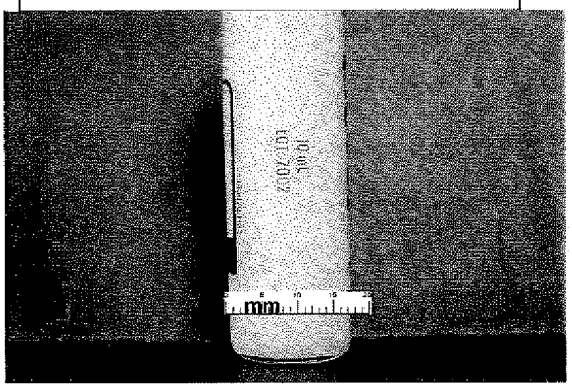
Example 1 – Immediately after shaking



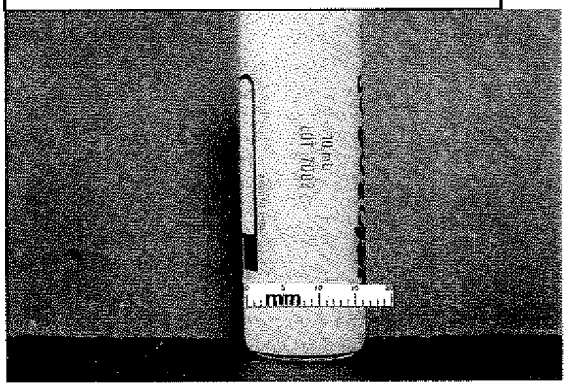
Example 1 – Initial Microscopy



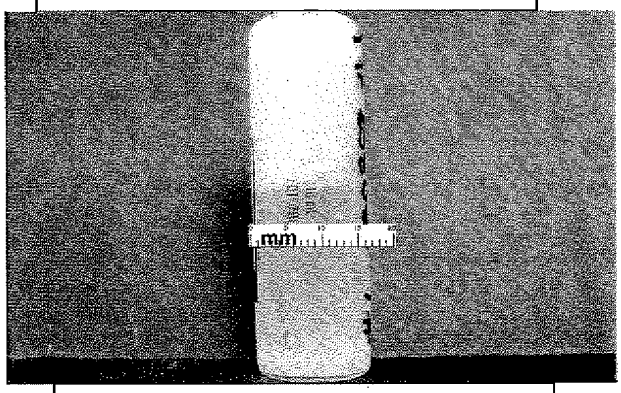
Example 1 – 30s after shaking



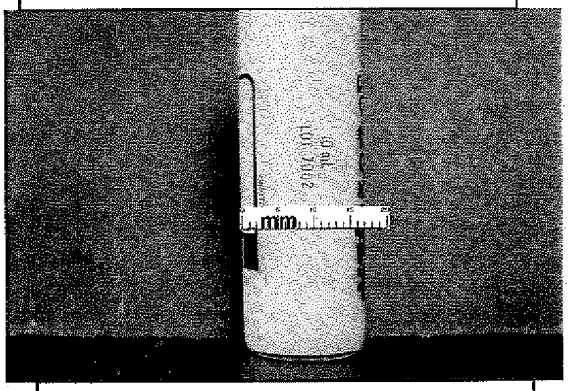
Example 5 – Immediately after shaking



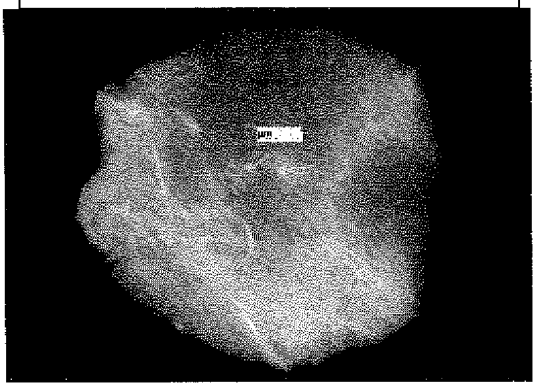
Example 1 – 5 min after shaking



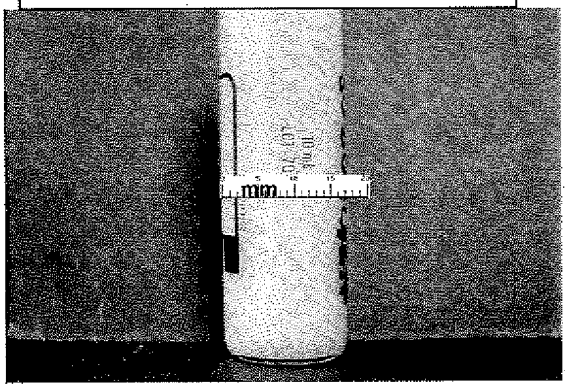
Example 5 – 30s after shaking



Example 1 – Initial Microscopy



Example 5 – 5 min after shaking



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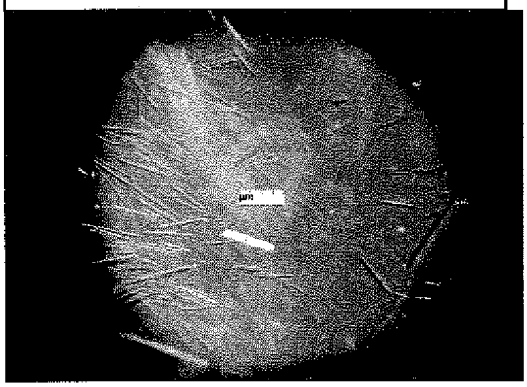


Example 5 – Initial Microscopy

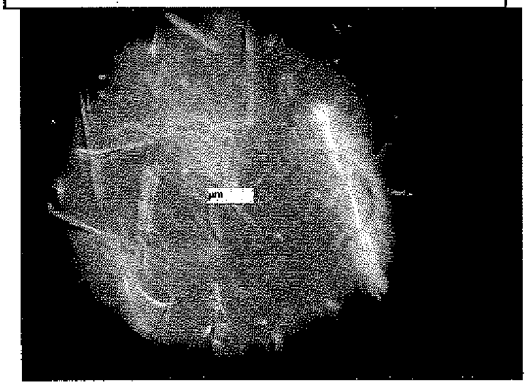
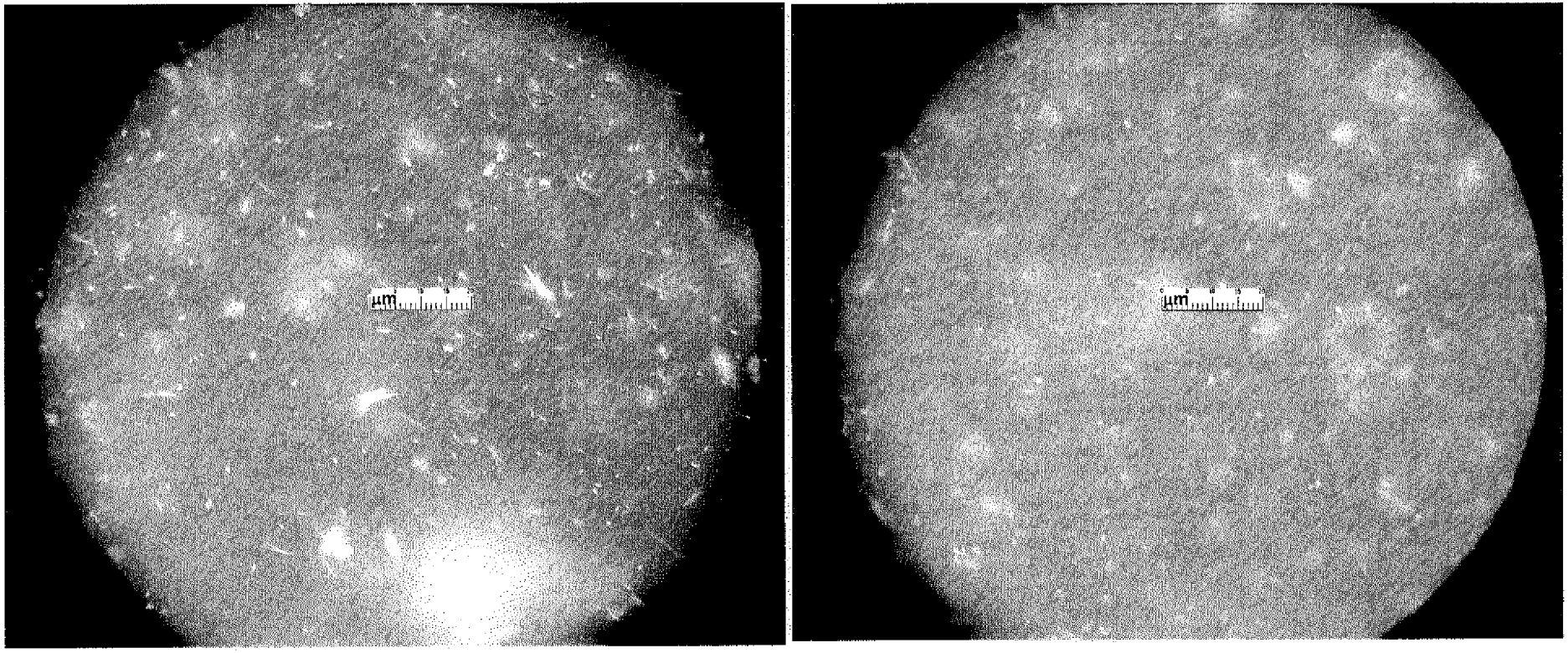


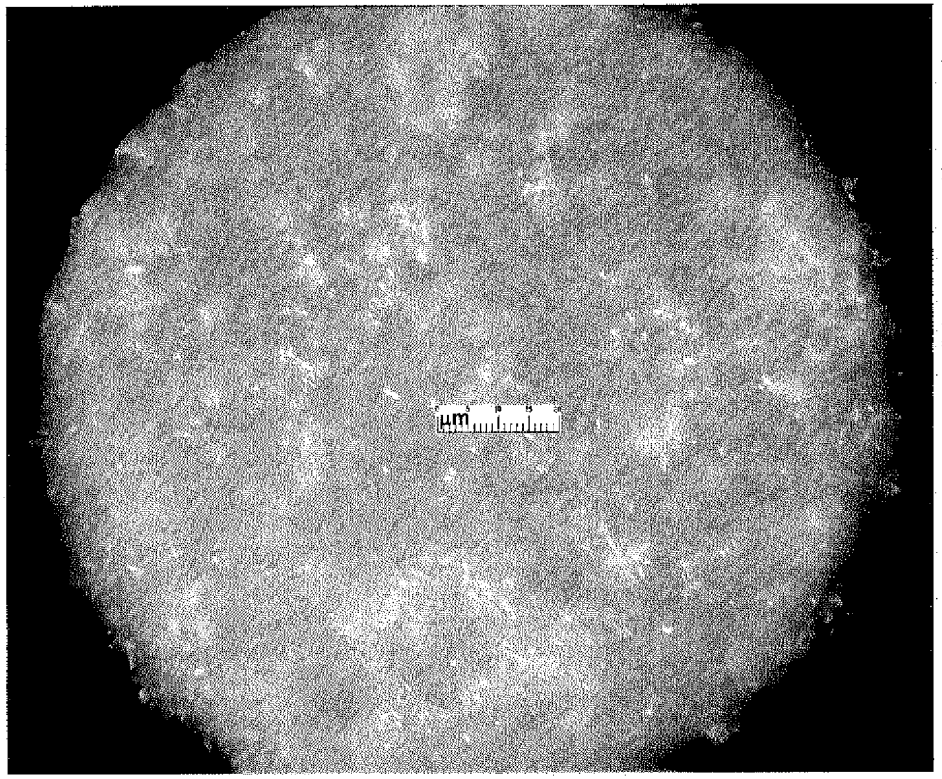
Plate 3

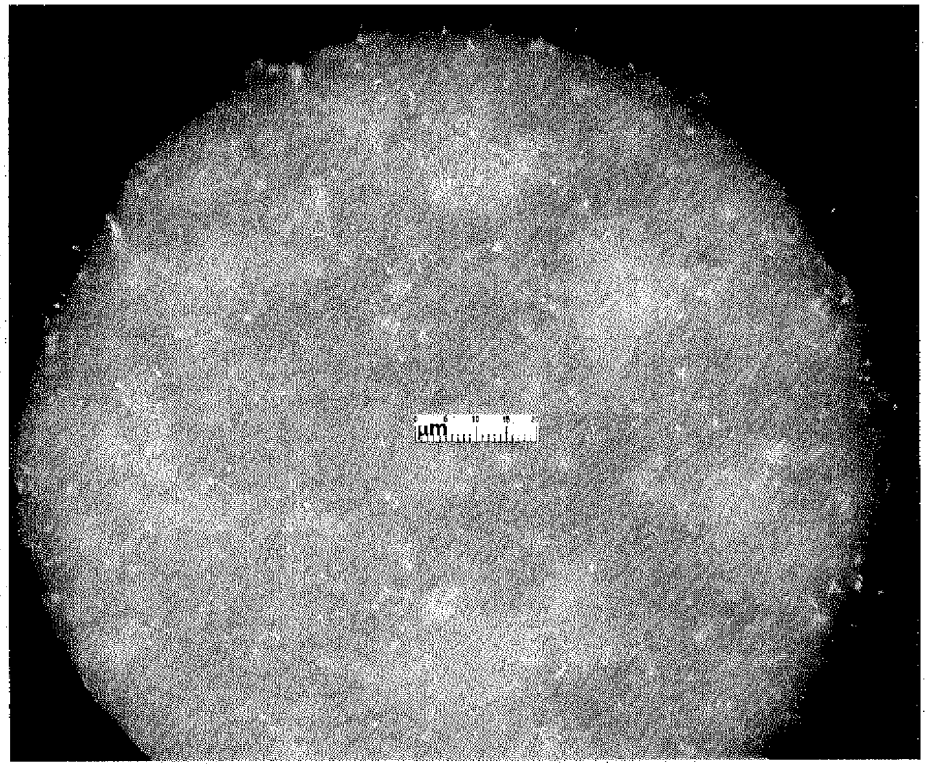
Attachment 2 to Declaration of David M. Anderson

EMBODIMENT 5: INITIAL MICROSCOPY

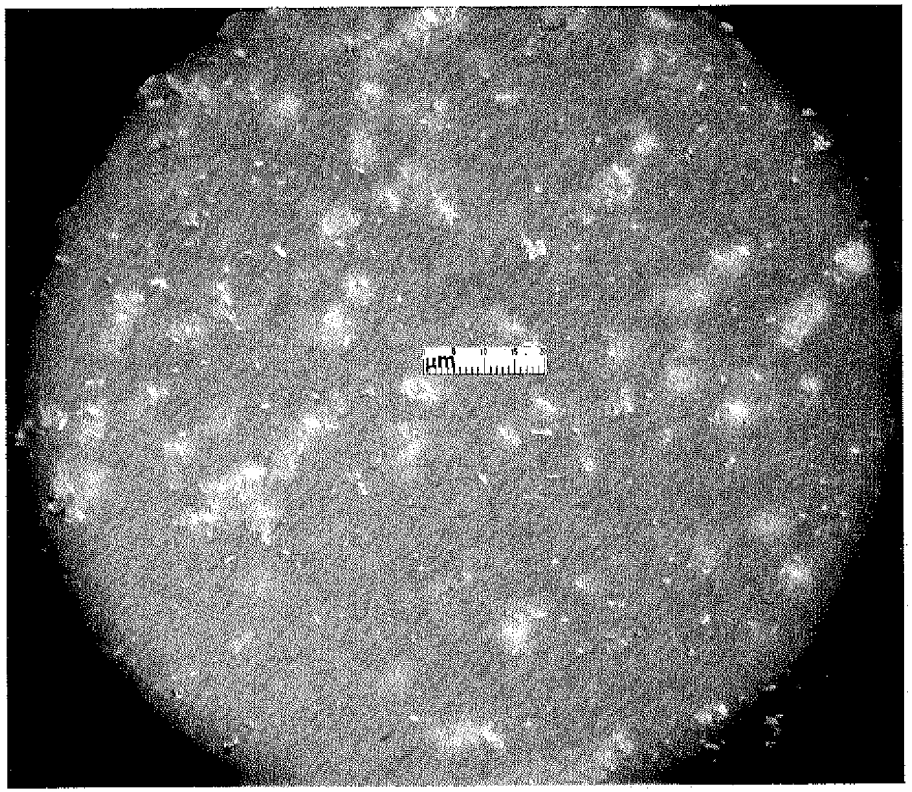


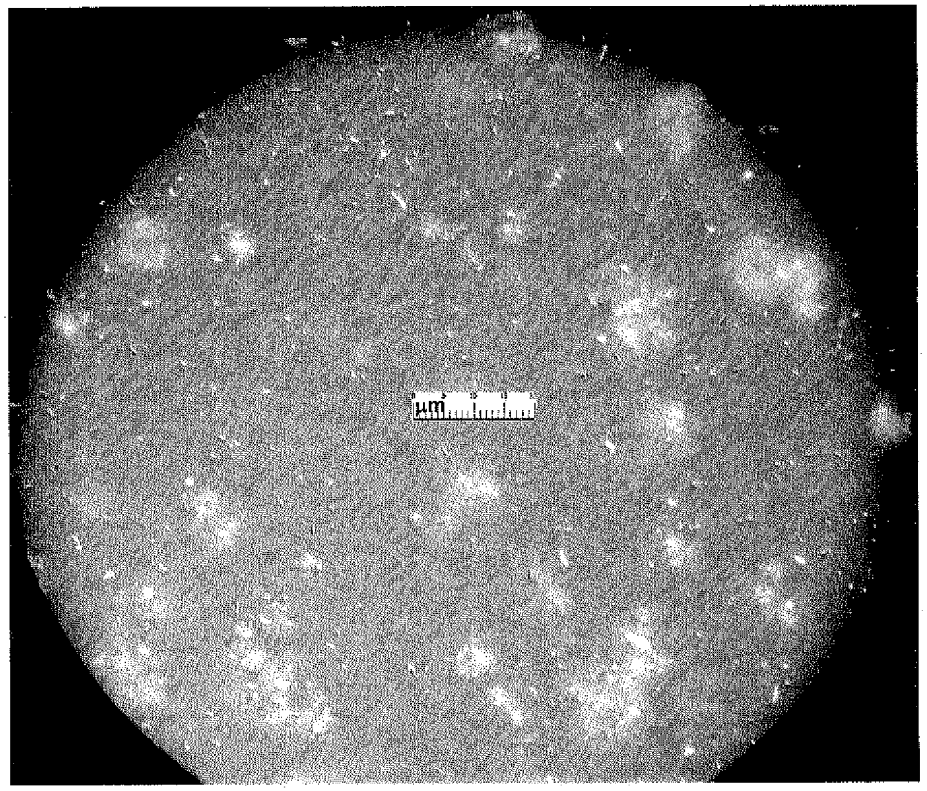
EMBODIMENT 5 AFTER 30 MINUTES HOMOGENIZATION



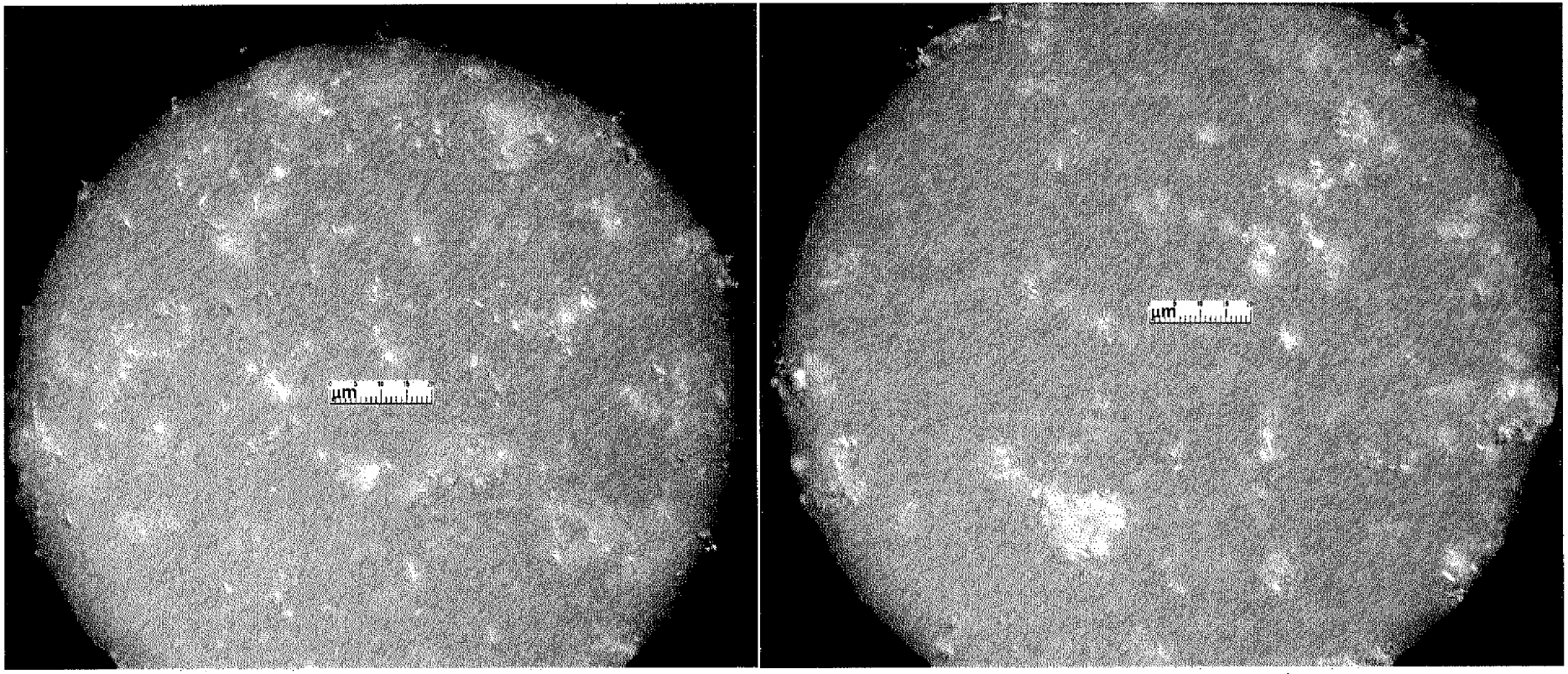


EMBODIMENT 5 AFTER 60 MINUTES HOMOGENIZATION





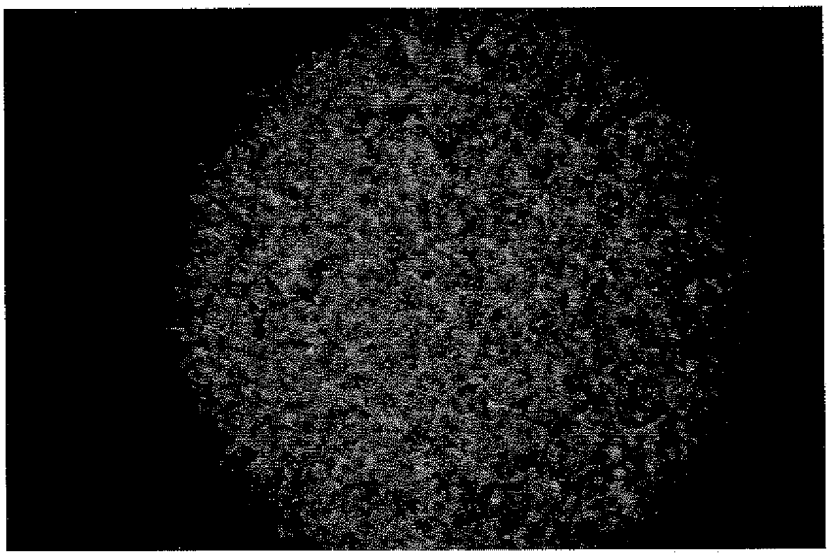
EMBODIMENT 5 AFTER 90 MINUTES HOMOGENIZATION

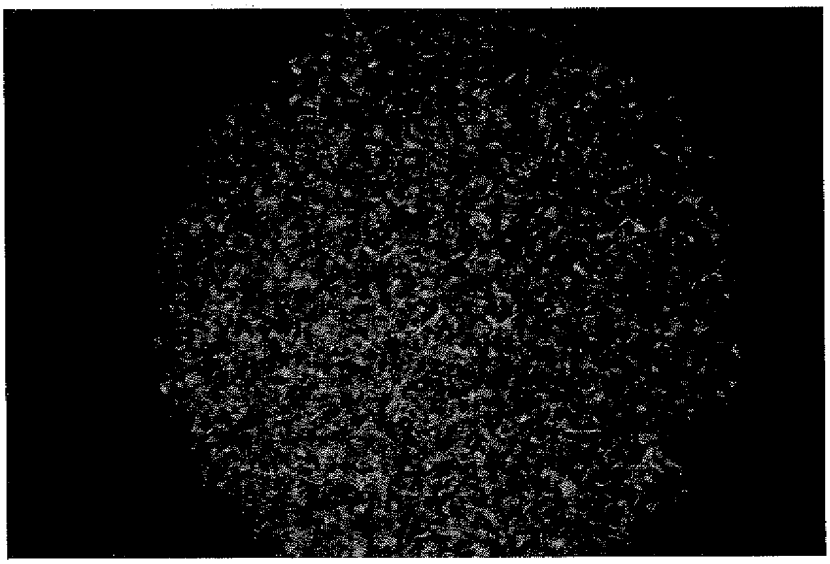


Attachment 3 to Declaration of David M. Anderson

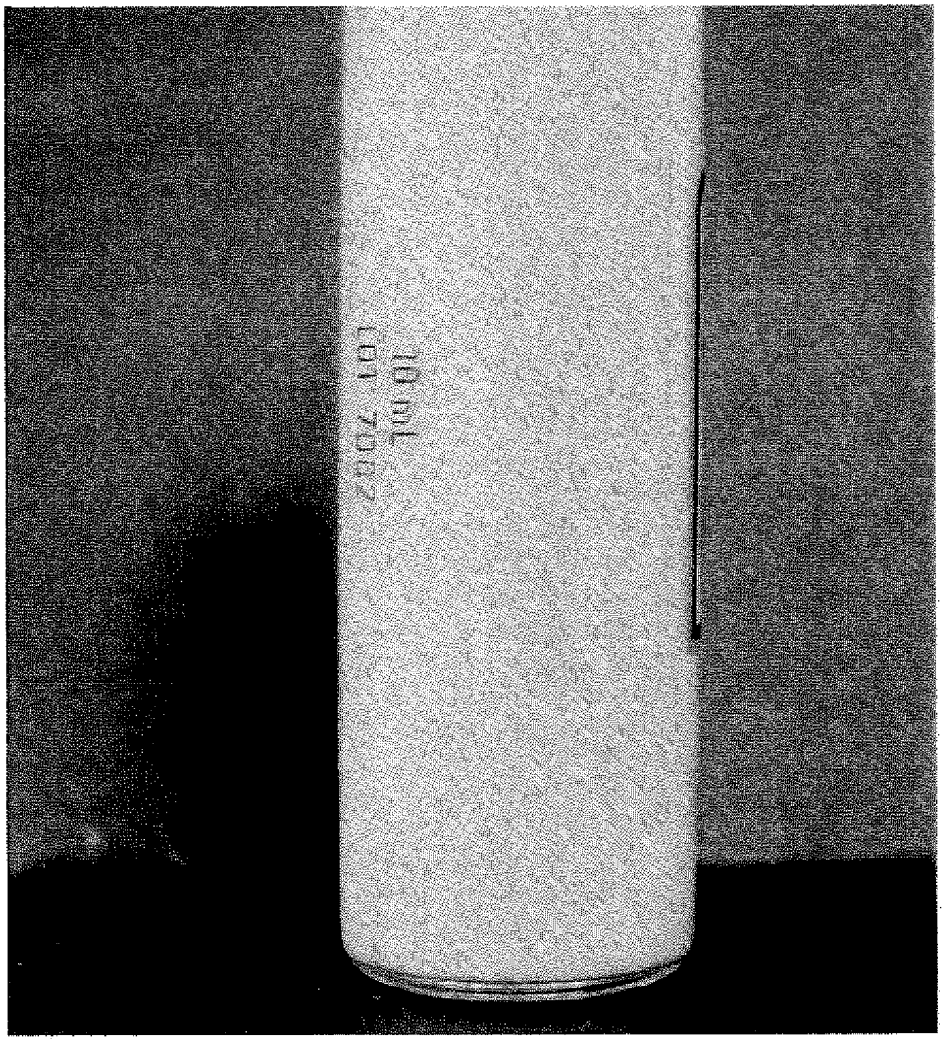
For assistance in evaluating the data regarding the formulations of the Japanese patent as set forth in Attachment 2, note the following with respect to a representative batch of a formulation of the Instant Invention.

These photomicrographs taken after only 15 minutes homogenization show no particles in excess of 5 microns. By contrast, even after 90 minutes homogenization Embodiment 5 of the Japanese patent continued to show apparently irreducible extremely large particles and aggregates. Ultrafine final mean particle size of approximately 220 nm is shown in micrographs of formulations of the Instant Invention in Attachment 4.





This photograph of a vial the above formulation after standing for 5 minutes after preparation shows no observable differentiation of regions, thus gives no indication whatsoever of settling or aggregating. By contrast, vials of Embodiments of the Japanese patent showed obvious differentiation of regions and in some cases frank sediment in this time or less as shown in Attachment 2.



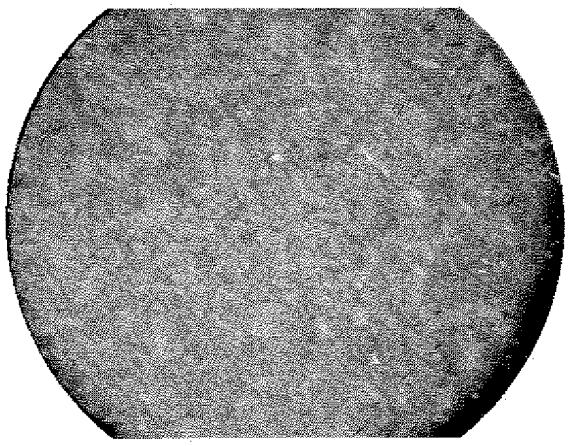
Attachment 4 to Declaration of David M. Anderson

In experimental work in the Applicants' laboratory, the "MC-NaD" formulation of Karan et al. 1996 was reproduced, and characterized for particle aggregation and growth.

Into a 250mL beaker, 0.99gm dantrolene sodium (Jinan Jinda, China), 0.99gm Lipoid E80 egg phosphatidylcholine (Lipoid GMBH, Ludwigshafen, Germany), and 150mL deionized water were added. This mixture was homogenized for 90 minutes with a Polytron PT3000 rotor-stator homogenizer. It was then microfluidized for 75 minutes in a Microfluidics M110-L microfluidizer at 14,000 psi.

The photomicrograph of the formulation immediately after microfluidization shows a significant population of needle-shaped crystals with a length in excess of 10 microns. The needles are obviously of a high aspect ratio (length to width ratio), being long in only one of three dimensions. Since laser particle sizing measures a "hydrodynamic diameter", which is effectively an average particle dimension, *even these 10 micron-long particles can be reported as only 1 or 2 microns in particle sizing*. This is the reason why my laboratory always uses a combination of laser particle sizing and DIC microscopy when determining the true particle size distribution of dantrolene dispersions. This is true in both the sodium dantrolene and free acid dantrolene cases. Although aggregation (clumping) of crystals is far less pronounced than in the next micrograph, some clumping is evident on close inspection, such as at the far right edge of the micrograph slightly above the horizontal midline.

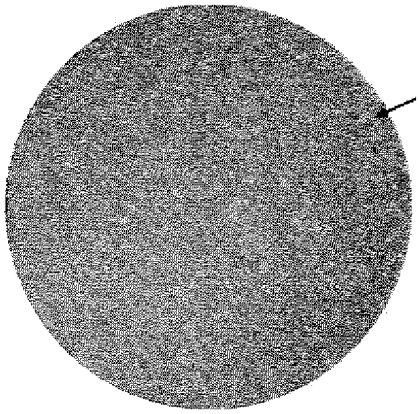
As the micrographs below show, when this formulation was examined only 1.5 hours after production, dramatic particle size growth and aggregation had already occurred, so much so that the material could no longer be run on the automated dynamic light scattering particle sizer instrument (Beckmann N4 Plus Photon Correlation Spectrometer). The micrograph shows a high density of individual needle-shaped crystals in excess of 25 microns in length. (The full width of the field of view at this magnification is approximately 150 microns). In addition, the micrograph shows a number of aggregates—clumps—of long crystals (see, for example, an "X" shaped clump along a line at 2 o'clock from the center of the field of view, about one-third of the way out toward the circumference), and such clumps are extremely dangerous for an intravenous product. In time, such clumps will develop into "bird's nests" of aggregated needles, which are certain to be life-threatening upon injection.



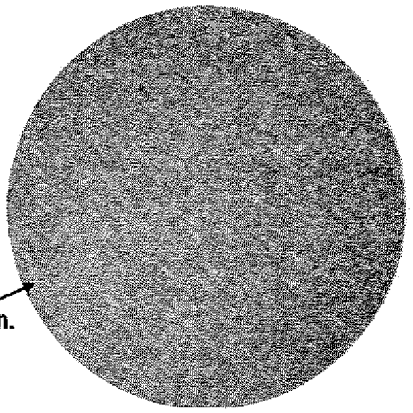
Karan MC-NaD formulation immediately after microfluidization.

Same formulation after 1.5 hours.

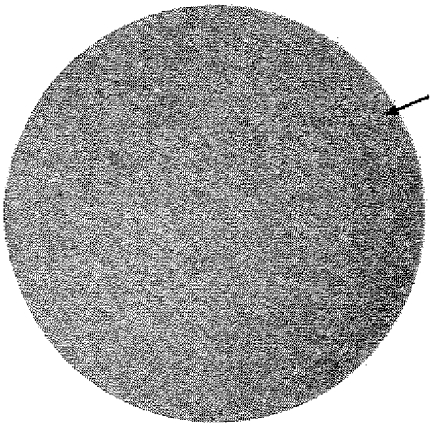
For comparison, below are photomicrographs of a formulation of the instant invention, showing that the formulation immediately after reconstitution is ultrafine, with a mean particle size 217 nm. The formulation maintains its ultrafine particle size and evidences no aggregation whatsoever for time periods many times as long as the MC-NaD above. The following optical micrographs show that at 7 hours after reconstitution, mean particle size is virtually identical, at 222nm mean particle size, as determined by dynamic light scattering (Beckman-Coulter N4 Plus Photon Correlation Spectrometer):



Immediately after reconstitution. Mean particle size 217 nm.



4 hours after reconstitution.



7 hours after reconstitution. Mean particle size 222 nm.